4-AMINOBIPHENYL

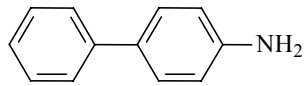
1. Exposure Data

1.1 Chemical and physical data

1.1.1 *Nomenclature*

Chem. Abstr. Serv. Reg. No.: 92–67–1
CAS Name: [1,1'-Biphenyl]-4-amine
Synonyms: 4-Amino-1,1'-biphenyl; 4-aminobiphenyl; *p*-aminobiphenyl; 4-aminodiphenyl; *para*-aminodiphenyl; biphenyl-4-ylamine; (1,1'-biphenyl-4-yl)amine; 4-biphenylamine; *para*-biphenylamine; 4-biphenylylamine; 4-phenylaniline; *para*-phenylaniline; 4-phenylbenzenamine; *para*-xenylamine; xenylamine

1.1.2 *Structural formula, molecular formula, and relative molecular mass*



$C_{12}H_{11}N$ Rel. mol. mass: 169.22

1.1.3 *Chemical and physical properties of the pure substance*

Description: Leaflets from alcohol or water (O'Neil, 2006); colourless to tan crystalline solid that turns purple on exposure to air (Pohanish, 2008)
Boiling-point: 302°C (Lide, 2008)
Melting-point: 53°C (O'Neil, 2006)
Solubility: Slightly soluble in cold water; readily soluble in hot water, acetone, chloroform, diethyl ether, and ethanol (O'Neil, 2006; Lide, 2008)
Octanol/water partition coefficient: log P, 2.80 (Verschueren, 2001)

1.1.4 *Technical products and impurities*

No information was available to the Working Group.

1.1.5 *Analysis*

The first analytical studies on 4-aminobiphenyl were reported during the 1960s. Method developments have enabled the detection of 4-aminobiphenyl at extremely low concentrations, down to the picogram level. GC/MS on derivatized samples and LC/MS on non-derivatized samples are most often used. Table 1.1 presents a selection of recent studies of the analysis of 4-aminobiphenyl in various matrices.

1.2 Production and use

1.2.1 *Production*

Because of its carcinogenic effects, 4-aminobiphenyl has not been produced commercially in the USA since the mid-1950s (Koss *et al.*, 1969). It was present in the drug and cosmetic colour additive D&C Yellow No. 1; however, use of this colour additive was discontinued in the late 1970s. 4-Aminobiphenyl also has been reported as a contaminant in diphenylamine (NTP, 2005; HSDB, 2009).

Available information indicates that 4-aminobiphenyl was produced and/or supplied in research quantities in the following countries: China, Germany, Hong Kong Special Administrative Region, India, Switzerland, and the USA (Chemical Sources International, 2008).

1.2.2 *Use*

4-Aminobiphenyl has been used formerly as a rubber antioxidant. It is reportedly still used in the detection of sulfates and as a model carcinogen in mutagenicity studies and cancer research (O'Neil, 2006).

1.3 Occurrence and exposure

1.3.1 *Natural occurrence*

4-Aminobiphenyl is not known to occur in nature.

1.3.2 *Occupational exposure*

Historically, occupational exposure to 4-aminobiphenyl mainly occurred during its production and its use as a rubber antioxidant and dye intermediate. No exposure measurements are available for these occupational exposure situations.

Occupational exposure can also occur in workers exposed to products contaminated with 4-aminobiphenyl, or in workers exposed to benzidine and benzidine-based dyes, from which 4-aminobiphenyl can be metabolically released (Lakshmi *et al.*, 2003; Beyerbach *et al.*, 2006). Other circumstances with potential exposure to 4-aminobiphenyl

Table 1.1. Selected methods of analysis of 4-aminobiphenyl in various matrices

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Finger paints	Paint containing amine is applied to an inert surface and dried. Painted sample and modifier (methanol) are placed in SFE cartridge for extraction.	SFE/GC	< 0.5µg/g	Garrigós *et al.* (1998, 2000)
Toy products	Supercritical fluid extraction (SFE), microwave-assisted extraction (MAE), or Soxhlet extraction with methanol	HPLC/UV	< 0.2µg/g	Garrigós *et al.* (2002)
Hair dyes	Isolation from dyes by solvent extraction with hexane, followed by silica gel chromatography, either with or without chemical treatment of the extract with zinc/HCl, and final purification with a mixed cation exchange reversed-phase resin	HPLC-ESI-MS	0.3ppb	Turesky *et al.* (2003)
Water	A mixture of 20 amines is dissolved in methanol, diluted to different concentrations for analysis. Other solvents are dichloromethane, ethyl acetate, and methanol/dichloromethane (50:50).	GC/MS	5ng/mL	Doherty (2005)
Dyes, cosmetics, inks and finger paints	Extract with methanol. Separate and detect on a phenyl ether linked stationary phase	HPLC/MS	40–500 pg	Hauri *et al.* (2005)
Urine	Acid hydrolysis of arylamine conjugates in urine, extraction with n-hexane, derivatization with pentafluoropropionic anhydride, and analysis	GC/MS	1ng/L	Riedel *et al.* (2006)

Table 1.1 (contd)

Sample matrix	Sample preparation	Assay method	Detection limit	Reference
Blood	Hydrolyse 4-aminobiphenyl-haemoglobin adduct using 1N sodium hydroxide. Purify and concentrate using solid phase extraction and elute on C-18 using chloroform. Evaporate and derivatize with pentafluoropropionic anhydride at room temperature. Dissolve in ethyl acetate for analysis	GC/MS	0.5 pg/g	Sarkar *et al.* (2006)
Air	Pre-concentration by percolating air through an acidic solution, ion-pair extraction with bis-2-ethylhexylphosphate (BEHPA), derivatization with isobutyl chloroformate (IBCF), and analysis	GC/MS	0.01 ng/m^3	Akyüz (2007)
Hair dye	Ion-pair extraction from aqueous samples with bis-2-ethylhexyl-prosphate released after solving the samples in acid solution, followed by sonication, derivatization of compounds with isobutyl chloroformate and analysis	GC/MS	0.02–0.2 ng/g	Akyüz & Ata (2008)

ESI, electrospray ionization; GC, gas chromatography; HPLC, high-performance liquid chromatography; MS, mass spectrometry; SFE, supercritical fluid extraction; UV, ultraviolet

include exposure to environmental tobacco smoke (Hammond *et al.* 1995), and exposure of laboratory workers working with 4-aminobiphenyl as a model in cancer research or for the detection of sulfate ions.

In workers exposed to benzidine or to benzidine-based dyes and a non-exposed control group in a dye factory in India, urine samples were analysed for 4-aminobiphenyl and acetylated 4-aminobiphenyl (Ac4ABP). 4-Aminobiphenyl was found in 30 of 33 urine samples from exposed workers (mean, 109 pmol/mL) and one of the 13 control workers. The benzidine-exposed workers had significantly higher 4-aminobiphenyl levels (57 pmol/mL) than did the benzidine-based dye-exposed workers (29.3 pmol/mL). In the sample that belonged to the person with the highest 4-aminobiphenyl concentration, acetylated 4-aminobiphenyl was found at a concentration of 79.5 pmol/mL, while it was not detected in any of the other urine samples (Beyerbach *et al.*, 2006).

1.3.3 *Environmental occurrence and exposure of the general population*

The main sources of exposure to 4-aminobiphenyl for the general population are cigarette smoking and environmental tobacco smoke. Other potential sources include emissions from cooking oils, hair dyes, and food and other dyes contaminated with it.

Living near benzidine-contaminated sites could entail exposure to 4-aminobiphenyl, as benzidine in the environment can be degraded to 4-aminobiphenyl by bacterial action (Bafana *et al.*, 2007).

(a) Occurrence in tobacco smoke and fumes from cooking oils

4-Aminobiphenyl is formed during tobacco combustion. Mainstream cigarette smoke was reported to contain 4-aminobiphenyl at levels of 2.4–4.6 ng per cigarette without a filter and 0.2–23 ng per cigarette with a filter; sidestream smoke contains up to 140 ng per cigarette (Patrianakos & Hoffmann 1979; Hoffmann *et al.*, 1997). A non-smoker exposed to environmental tobacco smoke during one month can ingest as much 4-aminobiphenyl as by smoking 17 cigarettes (Hammond *et al.,*1995)

4-Aminobiphenyl has also been detected in emissions from cooking oils. In a study from Taiwan, China, concentrations of 4-aminobiphenyl were 35.7 μg/m^3 in emissions from sunflower oil, 26.4 μ/m^3 from vegetable oil and 23.3 μg/m^3 from refined-lard oil (Chiang *et al.*, 1999).

(b) Occurrence as a contaminant

4-Aminobiphenyl can occur as contaminant in 2-aminobiphenyl, which is used in the manufacture of dyes. 4-Aminobiphenyl has also been detected in aniline (Reilly, 1967), in the drug and cosmetic colour-additive D&C Yellow No. 1 (HSDB, 2009) and in the food dye FD&C Yellow No. 6 (Richfield-Fratz *et al.*, 1985). 4-Aminobiphenyl also has been reported as a contaminant in diphenylamine, a fungicide used on apples (Olek, 1988).

4-Aminobiphenyl has been detected in hair dyes. In a study from the USA, Turesky *et al.* (2003) found 4-aminobiphenyl in eight of 11 different oxidative and direct hair

dyes, with levels of 4-ABP ranging from non-detectable (< 0.29 ppb) to 12.8 ppb. 4-Aminobiphenyl was found in blonde, red, and black, but not in brown dyes. The same study reported that research-grade 1,4-phenylenediamine (PPD), a key constituent for colour development of many permanent hair dyes, can be contaminated with 4-aminobiphenyl in concentrations up to 500 ppb. In a study from Turkey (Akyüz & Ata, 2008), 4-aminobiphenyl was found in concentrations up to 8.12 µg/g in 28 of the 54 hair-dye samples, up to 2.23 µg/g in 11 of 25 henna samples, and up to 2.87 µg/g in four of 10 commercial natural henna samples tested.

1,4-Phenylenediamine is manufactured by use of three methods: (1) reduction of *para*-nitroaniline, (2) diazotization of aniline, or (3) direct nitration of benzene without chlorinating. The major manufacturer of 1,4-phenylenediamine in the USA produces this chemical for use in hair dyes via the third process; it is > 99% pure.

1.4 Regulations and guidelines

1.4.1 *Europe*

(a) Council Directive 89/677/EEC

According to Council Directive 89/677/EEC, 4-aminobiphenyl may not be sold to the general public. The packaging shall be legible and indelibly marked as follows: "Restricted to professional users" (European Economic Community, 1989).

(b) Directive 98/24/EC

According to EU legislation, the manufacture of 4-aminobiphenyl has been prohibited since 1998 (European Commission, 1998). The Council Directive 98/24/EC in Annex III prohibits the production, manufacture or use at work of 4-aminobiphenyl and its salts and activities involving 4-aminobiphenyl and its salts. The prohibition does not apply if 4-aminobiphenyl and its salts are present in another chemical agent, or as a constituent of waste, provided that its concentration therein is less than 0.1% w/w.

(c) Directive 2002/61/EC

Directive 2002/61/EC restricts the marketing and use of azo-colourants (European Commission, 2002). In this Directive, Annex I to Directive 76/769/EEC is amended. Azodyes which, by reductive cleavage of one or more azo groups may release 4-aminobiphenyl in detectable concentrations, i.e. above 30 ppm in the finished articles or in the dyed parts thereof, according to the testing method established in accordance with Article 2a of this Directive, may not be used in textile and leather articles that may come into direct and prolonged contact with the human skin or oral cavity.

(d) Directive 2004/37/EC

4-Aminobiphenyl is regulated by the Directive 2004/37/EC (European Commission, 2004a), which applies to activities in which workers are exposed to carcinogens or mutagens of Category 1 and 2. Rules are fixed regarding the employers' obligations of reduction and replacement, prevention and reduction of exposure, unforeseen exposure, foreseeable exposure, access to risk areas, hygiene and individual protection, information for the competent authority, information and training of workers, consultation and participation of workers, health surveillance, record keeping and limit values.

(e) Cosmetics Directive

The Commission Directive 2004/93/EC of 21 September 2004 amends Council Directive 76/768/EEC for the purpose of adapting Annexes II and III thereto to technical progress (European Commission, 2004b). In this Directive, biphenyl-4-ylamine (i.e. 4-aminobiphenyl) and its salts are listed in Annex II as substances that must not form part of the composition of cosmetic products.

1.4.2 *Japan*

The Japan Society for Occupational Health (2007) follows the classification by IARC of 4-aminobiphenyl in Group 1 (IARC, 1987).

1.4.3 *Germany*

4-Aminobiphenyl is classified as a Category-1 carcinogen by the MAK Commission. The MAK Commission listed 4-aminobiphenyl as a substance for which percutaneous absorption may significantly contribute to systemic exposure. A MAK value was not set. A biological tolerance value (BAT) was set at 10.0 ng/L in blood, released from the 4-aminobiphenyl-haemoglobin-adduct (MAK, 2007).

1.4.4 *USA*

(a) ACGIH

4-Aminobiphenyl has been assigned an A1 notation, *Confirmed Human Carcinogen* (ACGIH, 2001). Accordingly, a numerical TLV (threshold limit value) is not recommended for occupational exposure. A ‘Skin notation’ is recommended (*potential significant contribution to the overall exposure by the cutaneous route*) as an additional precaution for undue exposure. As for all substances designated as A1 carcinogens without a TLV, workers should be properly equipped to eliminate all exposure to 4-aminodiphenyl to the fullest extent possible.

(b) NIOSH

The National Institute for Occupational Safety and Health lists 4-aminobiphenyl as one of thirteen OSHA-regulated carcinogens. Exposures of workers are to be controlled through the required use of engineering controls, work practices, and personal protective equipment, including respirators (NIOSH, 2005).

(c) FDA

FDA has set limits for 4-aminobiphenyl for the following certified colours (FDA, 1985, 1986, 1988): FD&C Yellow No. 5: ≤ 5 ppb; FD&C Yellow No. 6: ≤ 15 ppb; D&C Red No. 33: ≤ 275 ppb (FDA, 2009).

(d) NTP

4-Aminobiphenyl is listed in the NTP Report on Carcinogens as *known to be a human carcinogen* (NTP, 2005).

1.4.5 *Other*

(a) GESTIS

Table 1.2 presents some international limit values for 4-aminobiphenyl (GESTIS, 2007).

Table 1.2. International limit values (2007) for 4-aminobiphenyl

Country	Limit value – Eight hours		Limit value – Short-term	
	ppm	mg/m^3	ppm	mg/m^3
France	0.001	0.007		
Hungary				10
Italy				0.001

(b) Recent bans

The use and import of 4-aminobiphenyl were banned recently in the Republic of Korea in 2003, and in Switzerland in 2005 (UN/UNEP/FAO, 2007).

2. Studies of Cancer in Humans

2.1 Descriptive studies

In a case series, Melick *et al.* (1955) reported 19 cases of bladder cancer in 171 (11.1%) male workers engaged in the production of 4-aminobiphenyl. The exposure took place in a chemical plant in the USA between 1935 and 1955. In a later follow-up study, it was reported that among 315 male workers exposed to 4-aminobiphenyl, 53 had developed bladder tumours. The interval until development of bladder cancer varied from 15 to 35 years after beginning of the exposure (Melick *et al.*, 1971).

2.2 Cohort studies

Following the cessation of industrial production of 4-aminobiphenyl in 1955 in the USA, a large surveillance programme was started on workers reportedly exposed to this chemical. In the first study, a total of 601 specimens of urinary sediment from 285 men exposed to 4-aminobiphenyl were examined for cytological features. Among these men, 31 were found to have significantly abnormal epithelial cells in urinary sediments, of whom 10 were diagnosed with histologically confirmed bladder carcinoma (Melamed *et al.*, 1960). A later follow-up study was undertaken of the 22 patients whose urinary sediment in 1960 contained suspicious or frankly malignant cells, but did not have evidence of clinical bladder cancer. The study provided complete data on 18 patients, 11 of whom developed histologically confirmed carcinoma of the bladder (Koss *et al.*, 1965). In a third follow-up of this screening study, now extended to 503 male workers exposed to 4-aminobiphenyl, 35 workers developed histologically confirmed bladder cancer (Koss *et al.*, 1969). In a further extension, a group of 541 workers exposed to 4-aminobiphenyl were examined for abnormalities in cytologic specimens over a maximum period of 14 years. Among 86 men who had suspicious or positive cytology, 43 developed histologically confirmed carcinoma of the bladder (Melamed 1972).

Cancer mortality was studied among 884 male workers at the Nitro chemical plant in West Virginia (USA), which produced a variety of chemicals (Zack & Gaffey 1983). A ten-fold increase in mortality from bladder cancer was reported, with nine cases observed and 0.91 expected over the period 1955–1977. All nine cases started work in the plant before 1949; 4-aminobiphenyl was used in the plant from 1941 until 1952.

An updated and enlarged cohort study was conducted among workers at the same rubber chemicals plant in Nitro, West Virginia (USA) (Collins *et al.*, 1999). The aim of the study was to investigate any bladder cancer risk from exposure to 2-mercapto-benzothiazole (MBT). As 4-aminobiphenyl was shipped to the Nitro plant during 1935–1955 and used to produce a rubber antioxidant, MBT-exposed workers also had potential exposure to 4-aminobiphenyl. Exposure during the 20 years of manufacturing was limited to a few areas, e.g. where samples were taken for analysis, where distillation residues

were removed and where leaks occurred. The greatest exposure was found where workers were involved in repair of equipment and in cleaning-up of accidental leaks. As no information was available to determine which workers were involved in the clean-up of 4-aminobiphenyl, workers were considered to be exposed if they had a job in the department where 4-aminobiphenyl was used or were considered to have potential exposure to 4-aminobiphenyl if they were employed between 1935 and 1955, the years during which this chemical was used in the plant. The study examined the mortality of 1059 full-time white male hourly production workers employed at the rubber chemicals plant between 1955 and 1977; 600 of these workers were exposed to MBT. Follow-up was from 1955 to 1996. SMRs were computed by use of mortality rates for the white male population in the four counties in West Virginia within a 20-mile radius of the plant. For all study subjects, the SMRs for lung cancer, prostate cancer and other cancer sites were at expected levels. However, rates for bladder cancer were highly elevated in the total cohort (16 deaths, SMR, 6.3; 95% CI: 3.6–10.3), and in the subcohort of workers with exposure to 4-aminobiphenyl (eight deaths, SMR 27.1, 95% CI: 11.7–53.8). There was no excess of leukaemia, based on four deaths (SMR, 1.0; 95% CI: 0.3–2.6).

Cigarette smoking is an established cause of bladder cancer, and aminobiphenyls have been implicated in bladder-cancer etiology in smokers (IARC, 2004). Haemoglobin adducts of 4-aminobiphenyl are considered to be valid biomarkers of the internal dose of aminobiphenyl to the bladder (Probst-Hensch *et al.*, 2000). However, these biomarker studies have not been able to disentangle the role of 4-aminobiphenyl compared with other bladder carcinogens in tobacco smoke. Therefore, studies of tobacco smokers and bladder cancer are not reviewed here; the reader is referred to the Monograph on tobacco smoke (IARC, 2004).

3. Studies of Cancer in Experimental Animals

3.1 Oral administration

3.1.1 *Mouse*

A group of Ab × IF F_1 male and female hybrid mice, 10 weeks of age, were given commercial 4-aminobiphenyl (source and purity not stated) by gavage (0.2 mL of a 0.25% solution in arachis oil) twice weekly for 9 months. The estimated total intake was 38 mg per animal. Two of 12 mice surviving to 90 weeks developed bladder carcinoma. [The Working Group assumed there were none in 38 control animals.] Hepatomas were found both in treated animals and controls in similar frequencies (Clayson *et al.*, l965).

A group of C57 × IF F_1 male and female hybrid mice, 12 weeks of age, received 4-aminobiphenyl (Koch-Light Laboratories Ltd; purity not stated) by gavage (0.2 mL of a 0.25% solution in arachis oil) three times per week for 50 weeks. The estimated total intake was 75 mg per animal. The mice were killed approximately 20 weeks after the end

of the treatment. Thirteen of 28 (46%) female and four of 21 (19%) male mice had malignant liver tumours. One male mouse had a bladder carcinoma. In 50 control mice, one benign hepatoma was observed (Clayson *et al.*, 1967).

Groups of 840 male and 840 female BALB/cStCrlfC3Hf/Nctr mice from the NCTR breeding colony, four weeks of age, were given 4-aminobiphenyl-HCl (purity, > 99.5%) in the drinking-water at doses of 0, 7, 14, 28, 55, 110, and 220 ppm (males) and 0, 7, 19, 38, 75, 150, and 300 ppm (females). The 220-ppm and 300-ppm doses for one week corresponded to the LD_{50} (in mg/kg bw given by gavage) for male and female mice, respectively. Necropsies were performed at 13, 26, 39, 52, and 96 weeks, on approximately 45 organs and tissues of each animal. Induction of angiosarcomas (all sites), hepatocellular tumours and bladder carcinomas were dose-related (see Table 3.1) (Schieferstein *et al.*, 1985).

Table 3.1 Incidence of bladder carcinoma, hepatocellular carcinoma and angiosarcoma in BALB/c mice following chronic dosing with 4-aminobiphenyl in drinking-water

Sex	Dose (ppm)	Angiosarcoma (all sites)	Bladder carcinoma	Hepatocellular carcinoma
Female	0	1/119 (1%)	0/118 –	0/117 –
	7	4/120 (3%)	0/118 –	0/120 –
	19	4/120 (3%)	0/119 –	2/120 (2%)
	38	2/120 (2%)	1/118 (1%)	4/119 (3%)
	75	14/120 (12%)	0/118 –	10/119 (8%)
	150	26/118 (22%)	5/117 (4%)	14/118 (12%)
	300	11/117* (11%)	1/117 (1%)	7/117* (6%)
Male	0	1/118 (1%)	0/116 –	2/118 (2%)
	7	1/117 (1%)	1/117 (1%)	1/117 (1%)
	14	1/118 (1%)	1/118 (1%)	0/118 –
	28	2/119 (2%)	0/118 –	0/117 –
	55	4/115 (3%)	6/115 (5%)	0/114 –
	110	5/119 4%)	15/118 (13%)	3/118 (3%)
	220	14/118* (12%)	23/118* (19%)	2/117 (2%)

Adapted from Schieferstein *et al.* (1985)
*Positive trend, $P \leq 5.10^{-5}$

3.1.2 *Rabbit*

Seven rabbits (strain unspecified) were given 4-aminobiphenyl (source and purity not stated) orally (dose and dose regimen not given) to the limit of tolerance, and the treatment was continued until the onset of the final illness. Three animals were sacrificed in the first two years and two each between three and four years and between five and six years after the start of treatment. Bladder carcinomas were observed in three rabbits, the

earliest after four years of treatment. No tumours were observed in twelve control rabbits [no statistical analysis was provided] (Bonser, 1962).

3.1.3 *Dog*

Two male Beagle dogs, seven months of age, were given 4-aminobiphenyl (source and purity not stated; b.p. 302°C) in a gelatin capsule daily, six times per week until termination of the study. The dose level was lowered during the course of the experiment and dosing was also interrupted temporarily due to severe toxicity. The experiment was terminated after two years and nine months (total dose per dog: 2.9 and 3.3 g/kg bw, respectively); both dogs had bladder carcinoma [while no concurrent controls were included, historical data from this laboratory show that thirty Beagle dogs ranging from three to nine years of age did not develop bladder tumours] (Walpole *et al.*, 1954).

Four young adult female mongrel dogs were given a small quantity of 'purified' 4-aminobiphenyl (source not given) admixed into the food on five days a week for one year; after that, the animals received three times weekly by mouth a capsule containing 0.3 g of this compound. Bladder carcinomas were observed in all four dogs after 21–34 months. The total dose until first appearance of tumours was 87.5–144.0 g per dog, which corresponded to 8.2–14.1 g/kg bw (Deichmann *et al.*, 1958) [no controls were included].

Six female pure-bred beagle dogs, 6–12 months of age, were given 4-aminobiphenyl (source and purity not indicated) by mouth in a capsule at 1.0 mg/kg bw, five times per week for two years and 10 months (four dogs) or three years and one month (two dogs). The total dose range was 5.35–7.34 g per dog. Three bladder papillomas and three bladder carcinomas (transitional cell type) were observed in the six dogs (Deichmann *et al.*, 1965).

Two male and four female pure-bred beagle dogs were each given a single oral dose of 50 mg/kg bw 4-aminobiphenyl (purity, 98–99%) by capsule; the male dogs died within 18 hours, the females suffered severe acute intoxication, but survived and remained in good health for five years, as did two other female dogs that received a single oral dose of 15 mg/kg bw. There were no tumours in the urinary bladder of any of these female dogs (Deichmann & MacDonald, 1968). [The Working Group noted the inadequate dosing in this study.]

In an initiation-promotion study, four female pure-bred beagle dogs were given a single initiating dose of 4-aminobiphenyl (50 mg/kg bw; source and purity not specified) in corn oil by capsule. After recovery from severe acute methaemoglobinaemia, the dogs received a daily supplement of 6 g D,L-tryptophan as a promoter admixed into 300 g dog chow during the rest of the experiment. One dog killed due to injury after six months showed preneoplastic changes in the bladder of the type produced by tryptophan alone. After 4.5 years, the bladder of the three other dogs also showed typical changes seen after tryptophan treatment: macrophagic and lymphocytic infiltration, epithelial hyperplasia, irregularity of the basal layer and erosion of the luminal surface. One dog had developed a

small papillary bladder tumour. Since a single dose of 4-aminobiphenyl or chronic treatment with tryptophan had not produced tumours or preneoplastic changes in the bladder in previous studies (see Deichmann & MacDonald, 1968; Radomski *et al.*, 1971), this result was considered by the authors as suggestive of a promoting or co-carcinogenic effect of tryptophan (Radomski *et al.*, 1977). [The Working Group noted that the lack of a tryptophan-only treated group; the inadequate design of the Deichmann & MacDonald (1968) study made the authors' conclusion hard to believe.]

Twenty-four female pure-bred beagle dogs, four months of age, were given 4-aminobiphenyl (source, purity and dose (*sic*) not stated) in a corn oil suspension contained in a capsule on five days a week for three years. Twenty control animals were included. Two dogs remained without detectable tumours, two showed Grade-1 tumours very late in the study, and 20 animals developed tumours that ultimately progressed into Grade-2 and -3 bladder tumours (mainly transitional cell carcinomas) (Block *et al.*, 1978). [The Working Group noted the absence of information on the twenty control animals.]

3.2 Subcutaneous administration

3.2.1 *Mouse*

Fifty-two newborn Swiss mice were given a subcutaneous injection of 200 μg of 4-aminobiphenyl (Koch-Light; distilled before use) in 0.02 mL of 3% aqueous gelatin on each of the first three days of life. Forty-three mice survived until 48–52 weeks. Among the survivors, 19 of 20 (95%) male and 4 of 23 (17%) female mice had 'hepatomas'. Only three (3%) mice developed hepatomas in a control group of 42 males and 48 females (Gorrod *et al.*, 1968).

In a multistep in-vitro/in-vivo system, SV40-immortalized human urothelial cells were treated for 24h with 4-aminobiphenyl and maintained in culture for six weeks. The cells were then inoculated subcutaneously into female athymic nude (*nu/nu*) mice (age 4–6 weeks). After six months, 28 of 45 (62%) treated mice had developed carcinomas, and none of nine controls inoculated with cells treated with DMSO (Bookland *et al.*, 1992a).

3.2.2 *Rat*

Groups of 10 male and 12 female albino rats, three months of age and weighing 100 g, from a closed but randomly mated colony, were each subdivided in two groups and given 4-aminobiphenyl (purified, b.p. 302°C) in arachis oil by subcutaneous injection daily during 250–376 days, to a total dose of 3.6–5.8 g/kg bw. In the control group that received arachis oil only, one of 12 (8%) male and four of 11 (36%) female rats developed injection-site sarcomas. One female (9%) rat developed an intestinal tumour. In the treated groups, seven of 23 (30%) animals had intestinal tumours, one (4%) had a liver sarcoma, three of 12 females (25%) had mammary tumours and two (17%) had carcinoma of the uterus (Walpole *et al.*, 1952).

3.3 Intraperitoneal injection

3.3.1 *Mouse*

In a comparative carcinogenicity study of heterocyclic amines, newborn male B6C3F$_1$/nctr mice [initial number not clear] were given 4-aminobiphenyl (purity > 98%) by intraperitoneal injection. The amounts administered were 1.25 and 0.625 μmol dissolved in 35 μl dimethyl sulfoxide, injected in portions of 5, 10 and 20 μl on days 1, 8 and 15 after birth, respectively. Surviving pups were weaned on day 21 and designated for necropsy at 8 or 12 months of age. Mortality was 43% for the low-dose and 58% for the high-dose group. At 12 months, the 19 and 15 surviving mice in the low- and high-dose groups, respectively, all had liver adenomas (*vs* 11% in the 44 DMSO controls; $P < 0.001$, Fisher exact test). Among these, five mice in each group had liver carcinomas (26 and 33%, respectively) (Dooley *et al.*, 1992).

Twenty-four newborn B6C3F$_1$ mice were treated with 0.3 μmol 4-aminobiphenyl (re-purified to > 99%, verified by HPLC) dissolved in DMSO, by a series of intraperitoneal injections: 1/7th of the dose on postnatal day 1, 2/7th on day 8, and 4/7th on day 15. Eighteen control mice received DMSO only. Adenomas were found in 22.2% of the controls and in 79.2% of the treated mice. Hepatocellular carcinomas were observed in two treated animals (Parsons *et al.*, 2005).

Newborn male CD1 mice were injected intraperitoneally with 4-aminobiphenyl (purity > 99%) at a total dose of 625 nmol in 35 μl DMSO, given in portions of 5, 10 and 20 μl on days 1, 8, and 15 after birth, respectively. At weaning, the animals were divided into two groups, which were fed *ad libitum* until they were 14 weeks of age. Thereafter, one group received 90% of the calories of the *ad libitum* feeding regimen during one week, followed by 75% of the calories during one week, and then 60% of the calories in the diet until sacrifice at 12 months. Of 22 mice fed *ad libitum*, 12 (55%) had liver adenomas, six (27%) had hepatocellular carcinomas, and two mice (9%) had bronchio-alveolar adenoma. No liver tumours were seen in the calorie-restricted group ($n = 19$), but two mice (11%) developed bronchio-alveolar adenomas (Von Tungeln *et al.*, 1996). [The Working Group noted the lack of untreated controls.]

In a study to investigate the role of CYP1A2 in carcinogenesis induced by 4-aminobiphenyl, groups of mice deficient in cytochrome P450 1A2 (CYP1A2-*null*), derived from a mixed background of 129/Sv and C57BL/6 strains, received intraperitoneal injections of 10 and 20 μl of solutions of 4-aminobiphenyl (Aldrich; re-purified and re-crystallized) in DMSO on days 8 and 15 of age, respectively. One dose group was given a 20-mM solution and the other a 40-mM solution of 4-aminobiphenyl. The cumulative amounts of the test compound in the two dose groups were 600 and 1200 nmol, respectively. The mice were killed at 16 months of age and the livers analysed by histology. Mice proficient in CYP1A2 (CYP1A2+/+) were similarly treated. In the CYP1A2+/+ mice, both the high-dose and low-dose treatments caused a significant increase ($P < 0.01$) in liver adenomas (69–70%) and hepatocellular carcinomas (15–20%) in males compared with a control group consisting of a combination of CYP1A2+/+, +/–

and –/– mice treated with DMSO only. In females, the percentages were 3–17% and 0–4%, respectively. No differences in tumour incidence were seen with the different genotypes (CYP1A2-proficient or deficient) or with different doses of 4-aminobiphenyl (Kimura *et al.*, 1999). [The Working Group noted the inadequacy of the DMSO controls.]

3.4 Intravesicular implantation

Thirty-five albino mice [sex, age and strain not specified] received an intravesicular implant of 4-aminobiphenyl (British Drug Houses; dose unclear) in paraffin wax. After 40 weeks, three of 35 mice (9%) had developed an invasive bladder carcinoma. This tumour yield was not significantly different from that in the control mice implanted with paraffin wax alone (2/56; 4%) (Bonser *et al.*, 1956).

4. Mechanistic and Other Relevant Data

4.1 Absorption, distribution, metabolism, excretion

4.1.1 *Humans*

Grimmer *et al.* (2000) analysed the amounts of the aromatic amines 1- and 2-naphthylamine and 2- and 4-aminobiphenyl in the urine of 48 German smokers and non-smokers. Both groups excrete these four aromatic amines, with smokers excreting approximately twice as much as non-smokers (736 ng/24h *vs* 327 ng/24h). Similar amounts of urinary 2-naphthylamine and 4-aminobiphenyl were found in the two groups. The origin of the aromatic amines found in the urine of non-smokers is at present unknown. Based on the cotinine levels in the urine of non-smokers, environmental tobacco smoke can be excluded as a major source of aromatic amines. In addition, neither diesel exhaust-related nitroarenes nor the corresponding amino-derivatives to which they may be metabolically converted were detected. The aromatic amines in urine arising from sources other than tobacco smoke or diesel exhaust may play a role in the etiology of bladder cancer in non-smokers (Grimmer *et al.*, 2000).

A method for measuring *ortho*-toluidine, 2-naphthylamine and 4-aminobiphenyl in the urine of smokers and non-smokers used acid hydrolysis of the arylamine conjugates in urine, extraction with n-hexane, derivatization with pentafluoropropionic anhydride, and subsequent analysis with gas chromatography combined with mass spectrometry using negative-ion chemical ionization. The limits of detection were 4 ng/L for *ortho*-toluidine and 1 ng/L for 2-naphthylamine and 4-aminobiphenyl. Smokers ($n = 10$) excreted significantly higher amounts of *ortho*-toluidine (204 *vs* 104 ng/24 hours), 2-amino-naphthalene (20.8 *vs* 10.7 ng/24 hours), and 4-aminobiphenyl (15.3 *vs* 9.6 ng/24 hours) than non-smokers ($n = 10$). All non-smokers had quantifiable amounts of *ortho*-toluidine,

2-naphthylamine, and 4-aminobiphenyl in their urine, confirming that there are other environmental sources of exposure to these compounds (Riedel *et al.*, 2006).

4.1.2 *Experimental systems*

4-aminobiphenyl (4-ABP) is activated by cytochrome P450 (CYP), to produce the genotoxic *N*-hydroxy-4-ABP metabolite, which reacts with DNA.

Anderson *et al.* (1997) examined pancreatic tissues from 29 organ donors (13 smokers, 16 non-smokers) for their ability to metabolize aromatic amines and other carcinogens. Microsomes showed no activity in CYP1A2-dependent *N*-oxidation of 4-aminobiphenyl. Antibodies were used to examine microsomal levels of CYP1A2, 2A6, 2C8/9/18/19, 2E1, 2D6, and 3A3/4/5/7 and epoxide hydrolase. Immunoblots detected only epoxide hydrolase at low levels; CYP levels were < 1% of those in the liver. In pancreatic cytosols and microsomes, 4-nitrobiphenyl reductase activity was present at levels comparable to those in human liver. The *O*-acetyltransferase activity (AcCoA-dependent DNA-binding of radiolabelled *N*-hydroxy-4-aminobiphenyl) of pancreatic cytosols was high, about two thirds the levels measured in human colon. Cytosols showed high activity for *N*-acetylation of *para*-aminobenzoic acid, but not of sulfamethazine, indicating that acetyltransferase-1 is predominantly expressed in this tissue. Cytosolic sulfo-transferase was detected at low levels. ^{32}P-postlabelling showed putative arylamine-DNA adducts in most samples. In eight of 29 DNA samples, a major adduct was observed, which was chromatographically identical to the predominant ABP-DNA adduct, *N*-(deoxyguanosin-8-yl)-aminobiphenyl.

Chou *et al.* (1995) investigated the role of human sulfotransferase(s) in the bio-activation of the *N*-hydroxy metabolite of 4-aminobiphenyl (*N*-OH-ABP) *in vitro* with human tissue cytosols. Using an enzymatic assay consisting of a PAPS-regenerating system, radio-labelled *N*-OH-ABP, calf-thymus DNA and tissue cytosols, the sulfotransferase-mediated metabolic activation of *N*-OH-ABP was determined as the PAPS-dependent covalent binding of the *N*-OH substrate to DNA. The sulfotransferase(s) in human liver, and to a lesser extent colon, can readily metabolize *N*-OH-ABP. No metabolic activation was detected with cytosols prepared from human pancreas or from the carcinogen target tissue, the urinary bladder epithelium. The *N*-OH-ABP sulfotransferase activities of liver and colon cytosols from different individuals were highly correlated with their thermostable phenol sulfotransferase (TS-PST) activity (liver, $r = 0.99$, $P < 0.01$; colon, $r = 0.88$, $P < 0.01$). *N*-OH-ABP sulfotransferase activity was highly sensitive to inhibition by the selective TS-PST inhibitor 2,6-dichloro-4-nitrophenol ($IC_{50} = 0.7$ microM), and by *para*-nitrophenol. These data show that human liver TS-PST can metabolically activate the proximate human carcinogen *N*-OH-ABP to a reactive sulfuric acid ester intermediate that binds to DNA. In addition, in view of the putative role of *N*-OH-ABP as a major transport form of the carcinogen to the urinary bladder and of the absence of sulfotransferase activity in this tissue, sulfotransferase activation in the liver may actually decrease the bioavailability of *N*-OH-ABP to extrahepatic tissues and

thus serve as an overall detoxification mechanism for the urinary bladder (Chou *et al.*, 1995).

Adris and Chung (2006) investigated *Pseudomonas aeruginosa*, an opportunistic pathogen of the human urinary tract, and other selected human endogenous bacteria for metabolic activation of the bladder pro-carcinogens 2-aminofluorene, 4-aminobiphenyl, and benzidine. When incubated with each of those agents, the cell-free extracts of *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterobacter aerogenes*, *Proteus mirabilis*, *Proteus vulgaris*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Klebsiella pneumoniae*, and the intestinal anaerobes *Bacteroides fragilis*, *Clostridium perfringens*, and *Eubacterium aerofaciens* produced increased numbers of histidine revertants with the tester strain *Salmonella typhimurium* TA98 in the *Salmonella* mutagenicity assay. In addition, the cell-free extracts of *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Eubacterium aerofaciens* each showed the presence of a CYP absorption peak in the carbon monoxide difference spectrum. This was not observed with the other bacteria. These findings indicate that human endogenous bacteria, which are opportunistic pathogens of the urinary bladder, can metabolically activate these bladder pro-carcinogens into mutagens. The metabolic activation by *Pseudomonas aeruginosa*, *Bacteroides fragilis*, and *Eubacterium aerofaciens* is mediated by a CYP enzyme. For those organisms that induced metabolic activation but did not show a CYP absorption peak with the cell-free extracts, other oxidative enzymes may be involved.

4.1.3 *Other in-vitro studies*

In-vitro studies have been conducted with human and animal microsomal preparations of liver and urinary bladder, and with purified CYPs and recombinant CYPs (Beland & Kadlubar, 1985). CYP1A2 displays the highest catalytic acitivity of the CYPs for *N*-oxidation of 4-ABP, and it is the principal CYP involved in *N*-hydroxylation of 4-ABP in human and rat liver microsomes (Butler *et al.*, 1989). The k_{cat} of *N*-oxidation of 4-ABP is about fivefold greater with pooled human liver microsomes than with human urinary bladder microsomes fortified with NADPH, but the K_m values are comparable between the two microsomal preparations (Nakajima *et al.*, 2006). Several human endogenous bacteria, which are pathogens of the urinary bladder, can metabolically activate the 4-ABP and other bladder procarcinogens. For some of these microorganisms, metabolic activation is mediated by a CYP enzyme (Adris & Chung, 2006).

A large inter-individual variation in *N*-oxidation of 4-ABP in human liver microsomes is attributed to the variable CYP1A2 protein content (Butler *et al.*, 1989; Turesky *et al.*, 1998). Subjects who display relatively high CYP1A2 activity and who are rapid *N*-oxidizers of 4-ABP may be at elevated risk for cancer arising from exposure to aromatic amines or heterocyclic aromatic amines (Butler *et al.*, 1992). However, recent data from studies on laboratory animals have questioned the importance of CYP1A2 in the toxicity and carcinogenicity of 4-ABP. In *CYP1A2*-knockout animals, formation of methaemoglobin, which occurs by co-oxidation of *N*-hydroxy-4-ABP with oxy-

haemoglobin (Kiese, 1966), was lower in *CYP1A2*-knockout mice than in *CYP1A2*-wild-type mice (Shertzer *et al.*, 2002), and 4-ABP-DNA adducts were formed in livers of *CYP1A2*-knockout mice at even higher levels than in livers of *CYP1A2* wild-type mice (Tsuneoka *et al.*, 2003). In the neonatal mouse cancer model, *CYP1A2-null* mice had the same level of hepatocellular carcinoma (HCC) as the *CYP1A2*-wild-type mice following exposure to 4-ABP (Kimura *et al.*, 1999). From these findings, it appears that CYP1A2 is not the primary enzyme responsible for the *N*-hydroxylation 4-ABP in the mouse; thus other enzymes must be involved in the bioactivation and toxicities of 4-ABP. Indeed, liver microsomes from *CYP1A2-null* mice displayed an activity as least half as great as the liver microsomes from *CYP1A2*-wild-type mice in *N*-oxidation of 4-ABP (Kimura *et al.*, 1999). The enzymes responsible for this bio-activation remain to be determined. The high doses of 4-ABP employed in these animal studies could have triggered the activities of other enzymes to catalyse the *N*-oxidation of 4-ABP, a reaction process that normally does not occur after low-dose treatments. The *N*-oxidation of 4-ABP is known to be carried out principally by CYP1A2 in rat and human liver microsomes (Kimura *et al.*, 1999); these studies reveal species differences in 4-ABP metabolism by CYPs among mice, rats, and humans.

Extrahepatic CYPs, peroxidases, and prostaglandin-H synthase have been reported to catalyse the bioactivation of 4-ABP. Recombinant CYPs 1A1, 1B1 and 2A13 have been shown to bioactivate 4-ABP (5 μM), when induction of *umu* gene expression in *S. typhimurium* NM2009 was used as an endpoint (Shimada *et al.*, 1996; Nakajima *et al.*, 2006). These CYP enzymes could contribute to 4-ABP damage in extrahepatic tissues. Enzymes in bovine bladder epithelium and dog-bladder epithelium bioactivate arylamines, including 4-ABP (10–30 μg/plate) into bacterial mutagens in *S. typhimurium* TA98 (frame-shift specific) (Hix *et al.*, 1983), and they catalyse 4-ABP binding to DNA (Wise *et al.*, 1984). Intact cells or cell homogenates (S-9 fraction) from bovine bladder were found to be more effective than were liver preparations in the activation of 4-ABP (1–20 μg/plate) into a bacterial mutagen, in *S. typhimurium* TA98 (Hix *et al.*, 1983) and TA100 (point-mutation specific) strains (Oglesby *et al.*, 1983). Intact bovine urothelial cells also weakly bioactivated 4-ABP (5–20 μg/mL) into a mutagen in Chinese hamster V79 cells (Oglesby *et al.*, 1983). Prostaglandin H synthase, with arachidonic acid serving as a co-factor, has been shown to catalyse the binding of 4-ABP to DNA (Flammang *et al.*, 1989). This enzyme is present in urinary bladder epithelium, prostatic epithelium, colonic mucosa, and peripheral lung tissue of many species. Peroxidases, which are expressed at appreciable levels in the lung (Culp *et al.*, 1997) and mammary gland (Josephy 1996), also catalyse the binding of 4-ABP to DNA (Gorlewska-Roberts *et al.*, 2004). These results demonstrate the capacity of enzymes in extrahepatic tissues, including the bladder urothelium, to metabolically activate aromatic amines, and they suggest a role for the target organs in carcinogen bioactivation.

N-hydroxy-4-ABP can undergo further metabolism by phase-II enzymes to produce highly unstable esters. The esters undergo heterolytic cleavage to produce the reactive nitrenium ion, which readily forms adducts with DNA. NAT1 and NAT2 isoforms of

human and rodent *N*-acetyltransferases catalyse the formation of *N*-acetoxy-4-ABP (Minchin *et al.*, 1992), while sulfotransferase (SULT1A1) (Chou *et al.*, 1995) produces the *N*-sulfate ester of 4-ABP. These products may be the penultimate carcinogenic metabolites of 4-ABP formed *in vivo*. There are several metabolic reactions, including *N*-acetylation, that can compete with the CYP-mediated *N*-oxidation of 4-ABP. The resulting acetamide of 4-ABP is a poor substrate for CYP1A2, and *N*-acetylation is considered to be primarily a detoxification reaction (Cohen *et al.*, 2006). The rate of *N*-acetylation of 4-ABP with recombinant human NAT1 was threefold greater than with recombinant human NAT2, whereas the *O*-acetylation of *N*-hydroxy-4-ABP was about twofold higher with NAT2 (Minchin *et al.*, 1992). Thus, human NAT isoforms are involved in the detoxification of 4-ABP as well as in the bioactivation of *N*-hydroxy-4-ABP.

4.2 Genetic and related effects

4.2.1 *DNA adducts*

DNA adducts of 4-ABP have been reported *in vitro*, in bacterial and mammalian cells, in experimental animals exposed to 4-ABP or its metabolites, and in human tissues. Three major adducts are formed when *N*-hydroxy-4-ABP is reacted with calf-thymus DNA at pH 5.0: *N*-(deoxyguanosin-8-yl)-4-ABP (dG-C8–4-ABP) is the principal adduct and formed in 80% yield, followed by *N*-(deoxyadenosin-8-yl)-4-ABP (15% yield) and *N*-(deoxyguanosin-N^2-yl)-4-ABP (5% yield) (Beland *et al.*, 1983; Beland & Kadlubar, 1985). The N^2-deoxyguanosine adduct is unusual in that it contains a hydrazo linkage. More recently, 3-(deoxyguanosin-N^2-yl)-4-ABP and *N*-(deoxyguanosin-N^2-yl)-4-azobiphenyl have been identified as minor DNA adducts of 4-ABP (Hatcher & Swaminathan, 2002; Swaminathan & Hatcher, 2002).

(a) Humans

The adduct dG-C8–4-ABP was first detected by ^{32}P-postlabelling in biopsy samples of the human urinary bladder (Talaska *et al.*, 1991). Thereafter, the adduct was detected by gas-chromatography/negative-ion chemical ionization mass spectrometry (GC-NICI-MS) in mucosa specimens of human lung and urinary bladder; it was found at levels ranging from < 0.32–49.5 adducts per 10^8 nucleotides in lung, and from < 0.32–3.94 adducts per 10^8 nucleotides in the bladder samples (Lin *et al.*, 1994). A subsequent study reported the detection of the dG-C8–4-ABP adduct by immuno-histochemistry, ^{32}P-postlabelling, or GC-NICI-MS in bladder and lung tissue from smokers and ex-smokers (Culp *et al.*, 1997). The adduct levels measured by immuno-histochemistry ranged from three adducts per 10^8 nucleotides (the limit of detection) up to 505 adducts per 10^8 nucleotides. Comparable data were obtained through GC-NICI-MS measurements. The ^{32}P-postlabelling analyses underestimated the adduct level by up to 30-fold, suggesting that human lung DNA may be resistant to enzymatic hydrolysis,

possibly as a consequence of the high levels of DNA damage among current smokers. The 4-ABP adduct levels did not correlate with the numbers of cigarettes smoked per day or the duration of smoking, so the 4-ABP adducts in lung were proposed to originate from environmental exposure to 4-nitrobiphenyl (Culp *et al.*, 1997). The frequent detection of dG-C8–4-ABP adducts in non-smokers indicates that there may be other environmental sources of exposure as well: one such source may be hair dyes (Turesky *et al.*, 2003).

Wang *et al.* (1998) used immunohistochemistry to detect 4-ABP-DNA adducts in livers of subjects from Taiwan, China, with hepatocellular carcinoma (HCC). The mean relative staining intensity for 4-ABP-DNA was slightly higher in tumour tissues than in non-tumour tissues obtained from the same HCC patients. The mean intensities were significantly higher than the mean intensities from control tissues taken from non-HCC patients. However, no difference in mean relative staining intensity was found between smokers and nonsmokers in tissues obtained from non-HCC patients, or between tumour or non-tumour tissues taken from HCC cases. After stratification of the relative staining intensity data for 4-ABP-DNA adducts, there was a monotonically increasing risk for HCC with higher 4-ABP adduct levels; the linear relationship between adduct levels in liver tissue and HCC risk remained significant after adjustment for covariates, including hepatitis B surface-antigen status.

The DNA from the induced sputum of smokers, representing DNA of the lower respiratory tract, was shown to possess significantly higher levels of 4-ABP-DNA adducts than in the sputum of non-smokers, when assessed by immunohistochemical staining. The levels of adducts were related to indices of current smoking (cigarettes/day: $r = 0.3$, $P = 0.04$ and tar/day: $r = 0.4$, $P = 0.02$) (Besaratinia *et al.*, 2000). Faraglia *et al.* (2003) also detected 4-ABP-DNA adducts in female breast-tissue biopsy samples, when visualized by immunohistochemistry; the woman's smoking status was correlated with the levels of 4-ABP-DNA in tumour-adjacent normal tissues, but not in tumour tissue. 4-ABP-DNA adducts were also detected in laryngeal biopsies by immunohistochemical methods. Staining-intensity data showed a log-normal distribution, and values determined in tumour-adjacent tissue from individuals who smoked were significantly higher (median: 173.5, geometric mean: 159.9) than those values measured in tumour-adjacent tissue from non-smokers (median: 75.5, geometric mean: 7.40) (Faraglia *et al.*, 2003). Epithelial cell DNA isolated from human breast milk was shown by ^{32}P-postlabelling to contain *N*-(deoxyguanosin-8-yl)-4-ABP, and the adduct level was significantly associated with the use of hair-colouring products (OR = 11.2, 95% CI = 1.1–109.2), but not with tobacco usage (Ambrosone *et al.*, 2007).

Recent studies have employed liquid chromatography/electrospray ionization/tandem mass spectrometry (LC-ESI-MS/MS) methods to quantify dG-C8–4-ABP in human tissues. Zayas *et al.* (2007) detected dG-C8–4-ABP in urinary bladder epithelium in 12 of 27 subjects in DNA of tumour or non-tumour surrounding tissue. The level of adducts ranged from five to 80 adducts per 10^9 bases. The level of DNA adducts did not correlate with the level of the 4-ABP-haemoglobin-sulfinamide adduct; the latter is derived from 4-nitrosobiphenyl, an oxidation product of *N*-hydroxy-4-ABP, and a biomarker of exposure

to an internal dose of 4-ABP (Skipper & Tannenbaum, 1994; Zayas *et al.*, 2007). The lack of correlation of 4-ABP-DNA and -protein biomarkers may be attributed to an unknown time of formation of the observed adducts and to a variable persistence of dG-C8–4-ABP in the bladder. Because the activation or detoxification processes of 4-ABP metabolism as well as DNA-repair mechanisms may be tissue-specific, a correlation between haemoglobin (Hb) adducts and biopsied DNA adducts may not exist. The metabolism of 4-ABP is complex and/or may take place in one or more compartments that are distant from the urinary bladder. Thus, prediction of the relationship between exposure and adduct levels is not straightforward. Another pilot study reported the presence of dG-C8–4-ABP in six of 12 human pancreas samples (Ricicki *et al.*, 2005). The levels ranged anywhere from 1 to $60/10^8$ nucleotides, but there were no correlations among between the level of adducts, smoking preference, age, or gender. The frequency of hair-dye usage was not reported in the study.

4-ABP forms a major adduct at the 93β cysteine amino acid of human Hb (Green *et al.*, 1984; Ringe *et al.*, 1988). Adduct formation occurs following the co-oxidation reaction of *N*-hydroxy-4-ABP with oxy-Hb (Kiese 1966). The resulting 4-nitrosobiphenyl product reacts with the sulfhydryl group of cysteine to form a sulfinamide adduct (Green *et al.*, 1984; Ringe *et al.*, 1988; Bryant *et al.*, 1988). Several human biomonitoring studies have shown a relationship between the frequency of tobacco smoking, the extent of 4-ABP-Hb-adduct formation, and bladder-cancer risk (Bryant *et al.*, 1988; Skipper & Tannenbaum, 1994; Jiang *et al.*, 2007). Thus far, investigations have not examined the relationship between the frequency of hair dye-usage and 4-ABP-Hb adduct levels; however, statistically significant increases in levels of 3,5-dimethylaniline-Hb adducts have been found in women who regularly used permanent hair dyes (Gan *et al.*, 2004).

(b) Experimental systems

The methods of 4-ABP-DNA adduct detection include: HPLC with radiometric detection of tritium-labelled adducts; ^{32}P-postlabelling; immunhistochemistry; GC-NICI-MS of alkali-treated DNA, which cleaves the bond between the guanyl C8 atom and the 4-amino group of 4-ABP in dG-C8–4-ABP; and LC-ESI-MS/MS (Beland & Kadlubar, 1985; Lin *et al.*, 1994; Culp *et al.*, 1997; Doerge *et al.*, 1999).

N-Hydroxy-4-ABP reacts with DNA at neutral pH (pH 7.0) to form covalent adducts, but the reactivity is enhanced tenfold under acidic conditions (pH 5.0), which catalyses the generation of the highly reactive nitrenium ion (Beland *et al.*, 1983). Such mildly acidic pH conditions can occur in the urinary bladder and can cause the hydrolysis of the metabolite N^4-(β-glucosiduronyl)-*N*-hydroxy-4-ABP to produce *N*-hydroxy-4-ABP and its nitrenium ion, leading to DNA adducts in the bladder epithelium (Beland *et al.*, 1983; Kadlubar *et al.*, 1991).

Beagle dogs were treated with tritium-labelled 4-ABP at a single dose of 60 µmol/kg bw and sacrificed after one, two, or seven days. DNA adducts in urothelial cells were monitored by HPLC with radiometric detection (Beland & Kadlubar, 1985). The level of adducts in the animals was essentially constant at each time point, with

N-(deoxyguanosin-8-yl)-4-ABP accounting for 76% of the total binding. The next most abundant product was the N^2-deoxyguanosine substituted adduct (15%), followed by the *N*-(deoxyadenosin-8-yl)-4-ABP adduct (9%). These same adducts and 3-(deoxyguanosin-N^2-yl)-4-aminobiphenyl and *N*-(deoxyguanosin-N^2-yl)-4-azobiphenyl were detected by ^{32}P-postlabelling of DNA in human uroepithelial cells exposed to *N*-hydroxy-4-ABP (Hatcher & Swaminathan, 2002; Swaminathan & Hatcher, 2002).

DNA adducts of 4-ABP were quantified, by ^{32}P-postlabelling and immunohistochemistry, in liver and bladder of male and female BALB/c mice, following treatment with a range of concentrations of 4-ABP (0, 7, 14, 28, 55, 110 or 220 ppm; male) and (0, 7, 19, 38, 75, 150 or 300 ppm; female) in the drinking-water for 28 days (Poirier *et al.*, 1995). The principal adduct in both tissues, for both genders, was dG-C8–4-ABP. A comparison between DNA-adduct formation and tumourigenesis indicated a linear correlation between adduct levels and incidence of liver tumours in female mice. The relationship between adducts and tumourigenesis was distinctly nonlinear in the bladders of male mice, and tumour incidence rose rapidly above the 50-ppm dose of 4-ABP: toxicity and cell proliferation may have increased the bladder-tumour incidence.

4.2.2 *Mutations and other related effects*

(a) Mutagenesis in bacteria

4-Aminobiphenyl and other primary arylamines are frameshift mutagens in *S. typhimurium* strain TA1538 (Beland *et al.*, 1983). When revertants were expressed as a function of DNA binding, *N*-hydroxy-*N*′-acetylbenzidine was found to be the most mutagenic arylamine, followed by *N*-hydroxy-2-aminofluorene, and then by *N*-hydroxy-2-naphthylamine (*N*-OH-2-NA) and *N*-hydroxy-4-aminobiphenyl; the latter two showed approximately the same number of revertants per adduct. In a similar study conducted with *S. typhimurium* strain strain TA 1535, which detects base-substitution revertants, only *N*-OH-2-NA induced mutations (Beland *et al.*, 1983).

More recent studies with the TA100 strain (base substitution-specific) containing plasmid pKM101 (which enhances the mutagenic potential of some genotoxicants through error-prone DNA-repair polymerases) have shown that 4-ABP (3–300 µg/plate) induces revertants in the TA100 strain, in the presence of a rat liver S-9 mix from animals pretreated with polychlorinated biphenyls (PCBs), at levels five- to tenfold higher than in TA98 (frameshift-specific) (Chung *et al.*, 2000). The mutagenic potency of 4-ABP (1–1000 µg/plate) was also greater in strain YG1029 (base substitution-specific strain), which has multiple copies of the bacterial *O*-acetyltransferase (Dang & McQueen, 1999), indicating formation of the reactive *N*-acetoxy-4-ABP intermediate.

The induction of the *umu* response of 4-ABP (0.1–20 µM) in *S. typhimurium* strain NM6001, containing human NAT1, was about fourfold greater than in strain NM6002 expressing human NAT2, when recombinant human CYP1A2 was used for bioactivation of 4-ABP (Oda, 2004). Consistent with these data, NAT1 was superior to NAT2 in inducing the binding of *N*-hydroxy-4-ABP to DNA (Minchin *et al.*, 1992). 4-ABP

(5 μg/plate), in the presence of rat liver S-9 (PCB pretreatment), also induced mutations in *S. typhimurium* strain TA102, which is sensitive to agents producing reactive oxygen species (Makena & Chung, 2007). The generation of reactive oxygen species may occur through redox cycling of *N*-hydroxy-4-ABP and its nitroso metabolite (Kim *et al.*, 2004) via a hydronitroxide radical as an intermediate (Makena & Chung, 2007).

Verghis *et al.* (1997) studied mutagenesis of 4-ABP in *E.coli* in the *lacZ* gene using M13 cloning vectors able to detect many base-pair substitution mutations and most insertions and deletions. To minimize the effect of host DNA-repair processes, mutagenicity experiments were performed with single-stranded DNA randomly modified with 4-ABP instead of double-stranded DNA. Sequence analysis of 4-ABP-induced mutations in the *lacZ* gene revealed exclusively base-pair substitutions, with over 80% of the mutations occurring at G sites. Among the sequence changes at G sites, G:C → to T:A transversions predominated, followed by G:C → C:G transversions, and G:C → A:T transitions. An oligonucleotide containing the major DNA adduct, *N*-(deoxyguanosin-8-yl)-4-aminobiphenyl (dG-C8–4-ABP), was situated within the *Pst*I site of a single-stranded M13 genome to examine the mechanism of genotoxicity of this adduct. After *in vivo* replication of the adduct-containing 4-ABP-modified and control (unadducted) genomes, the mutational frequency and mutational specificity of the dG-C8–4-ABP lesion were determined. The targeted mutational efficiency was ~0.01%, and the primary mutation observed was the G:C → C:G transversion (69%), followed by G:C → to T:A transversions (23%). In the mutagenesis studies with the randomly 4-ABP-modified vector, G:C → T:A transversions were the most numerous, possibly reflecting a sequence-context effect for the dG-C8–4-ABP lesion. Thus, although dG-C8–4-ABP is weakly mutagenic at the *Pst*I site, it can contribute to the mutational spectrum of 4-ABP lesions (Verghis *et al.*, 1997).

(b) Mutagenesis in mammalian cells

(i) *Mutations in* HGPRT*, chromosomal instability*

4-Aminobiphenyl and three of its proximate *N*-hydroxylated carcinogenic metabolites (0.5–400 μM) were mutagenic in an SV40-immortalized human uroepithelial cell line, as assayed by use of induction of mutations in the hypoxanthine-guanine phosphoribosyl-transferase (*HGPRT* or *HPRT*) locus (Bookland *et al.*, 1992b). 2-ABP did not induce mutations in this cell line at these concentrations.

Human bladder-cancer (RT112) cells treated with 4-ABP (125 μg/ml), with rat liver S-9 (after pretreatment with PCB) used for bio-activation, developed chromosomal instability in 50–60% of the cells. 4-ABP could affect tumour occurrence by inducing chromosomal instability, which represents the predominant form of genetic instability in human solid tumours (including those in bladder and colon) (Saletta *et al.*, 2007).

N-Hydroxy-acetylaminobiphenyl (*N*-OH-AABP) at a range of doses (0.5, 1.0, and 10.0 μM) induced mutations by up to five- and 16-fold over background levels,

respectively, at the *HPRT* and thymidine kinase (*TK*) loci of human lymphoblastoid TK6 cells (Luo *et al.*, 2005; Ricicki *et al.*, 2006).

(ii) *H*-ras *mutations caused by 4-ABP*

DNA adducts and mutations were induced in the livers of neonatal wild-type B6C3F$_1$ and CD-1 mice, and in Big Blue B6C3F$_1$ transgenic neonatal and adult mice treated with a regimen of 4-ABP known to induce tumours in neonatal mice (total doses: 3, 9 or 31 mg/kg 4-ABP at days 8 and 15 postnatal; 31 mg/kg bw for adults) (Manjanatha *et al.*, 1996). dG-C8–4-ABP was the major DNA adduct identified in the livers of Big Blue B6C3F$_1$ mice. Adduct levels for adult females were about twofold higher than for neonatal females, while adduct levels in adult males were less than half those in neonatal males. In the neonatal wild-type B6C3F$_1$ mice, 4-ABP preferentially induced C:G → A:T mutations (reflecting G:C → T:A transversions in the non-coding strand) in H-*ras* codon 61, followed by G:C → C:G mutations. However, in CD-1 mice, 4-ABP primarily induced A:T → T:A transversions in H-*ras* codon 61, a molecular feature consistent with the formation of the *N*-(dA-C8-yl)-4-ABP adduct (Manjanatha *et al.*, 1996). In the C57B1/10J mouse strain, mutations in codon 61 of the H-*ras* gene were not significantly implicated in chemical induction of liver tumours by 4-ABP (ABP was administered in diet at 200 and 600 ppm for nine months) (Lord *et al.*, 1992), suggesting that the incidence of *Ras* mutations in chemically-induced mouse liver tumours is strain-dependent.

(iii) *Transgene mutations caused by 4-ABP*

4-ABP was evaluated in the Muta^{TM}Mouse transgenic mouse mutation assay. A single oral dose of 75 mg/kg bw induced, respectively, 6.9-, 1.8- and 2.2-fold increases in the mutation frequency (MF) in the bladder, liver and bone marrow. Ten daily oral doses of 10 mg/kg body weight of 4-ABP increased the MF in the bladder, liver, and bone marrow by 13.7-, 4.8- and 2.4-fold the control value, respectively (Fletcher *et al.*, 1998).

4-ABP treatment significantly increased the mutations in the liver *cII* transgene in both genders of neonatal Big Blue B6C3F$_1$ transgenic mice but not in the adult mice, following treatment with a regimen of 4-ABP known to induce tumours in neonatal mice (total doses of 3, 9 or 31 mg/kg 4-ABP at days 8 and 15 postnatal; 31 mg/kg bw for adults). Sequence analysis of *cII* mutant DNA revealed that 4-ABP induced a unique spectrum of mutations in the neonatal mice, characterized by a high frequency of G:C → T:A transversions, while the mutational spectrum in 4-ABP-treated adults was similar to that in control mice. These authors suggested that neonates are more sensitive than adults to 4-ABP, because the relatively high levels of cell division in the developing animal facilitate the conversion of DNA damage into mutations (Chen *et al.*, 2005). The mutational spectrum data are consistent with the data seen for the tetracycline resistance gene of the plasmid pBR322, where 4-ABP modification resulted in G:C → to T:A and G:C →C:G transversions; frameshift mutations occurred with a lower frequency (Melchior *et al.*, 1994).

N-Hydroxy-4-acetylaminobiphenyl (*N*-hydroxy-AABP) (10–320 μM) caused a dose-dependent increase in mutation frequency – up to 12.8-fold over the background level – in the *cII* transgene in embryonic fibroblasts of the Big Blue mouse (Besaratinia *et al.*, 2002). A high frequency of G:C → T:A transversions occurred in the *cII* transgene of treated cells. The authors concluded that the pattern of mutations induced in the *cII* gene by this metabolite of 4-ABP is at odds with the mutational spectrum of the *TP53* gene in human bladder cancer, where G:C → A:T transitions are the dominant type of mutation (Olivier *et al.*, 2002).

(iv) TP53 *mutations induced by 4-ABP*

About 50% of bladder cancers contain a mutation in the tumour-suppressor gene *TP53* (Olivier *et al.*, 2002). The spectrum of mutations in the *TP53* gene in smokers and non-smokers with bladder cancer shows base substitutions occurring at G:C and A:T base pairs. Codon 285 of the *TP53* gene, a mutational hotspot at a non-CpG site in bladder cancer, was found to be the preferential binding site for *N*-hydroxy-4-ABP (30 μM) *in vitro* (Feng *et al.*, 2002b). Moreover, C5-cytosine methylation greatly enhanced *N*-hydroxy-4-ABP binding at CpG sites; two mutational hotspots at CpG sites, codons 175 and 248, became preferential binding sites for *N*-hydroxy-4-ABP only after being methylated. The distribution of 4-ABP–DNA adducts was mapped in the *TP53* gene at the nucleotide-sequence level in human bladder cells (HTB-1) treated with *N*-hydroxy-4-ABP or *N*-hydroxy-AABP (30 μM) and mutational hotspots in bladder cancer at codons 175, 248, 280, and 285 were preferential sites for 4-ABP adduct formation (Feng *et al.*, 2002a). The authors suggested that 4-ABP contributes to the mutational spectrum in the *TP53* gene in human bladder cancer. These data provide some molecular evidence that links 4-ABP to bladder cancer, but the roles of methylation status and transcriptional activity in the mutational spectrum in the *TP53* gene induced by 4-ABP have yet to be determined.

4.2.3 *Other biological effects of 4-ABP in mammalian cells*

In mammalian cells, 4-ABP and three of its proximate *N*-hydroxylated carcinogenic metabolites (0.5–400 μM) were genotoxic in an SV40-immortalized human uroepithelial cell line, in which mutation induction was measured at the hypoxanthine-guanine phosphoribosyl-transferase (*HGPRT*) locus (Bookland *et al.*, 1992b). The isomeric 2-ABP did not induce mutations at these concentrations. At very high concentrations, 2-ABP and 4-ABP (500 μM), among other arylamines, were reported to induce DNA damage in human lymphocytes, as revealed by use of the comet assay (Chen *et al.*, 2003).

With *N*-hydroxy-AABP at a range of doses (0.5, 1.0 and 10.0 μM), the levels of dG-C8–4-ABP adducts in the cells ranged from 18 to 500 adducts per 10^9 nucleotides at 27 hours, and resulted, respectively, in 95%, 85%, and 60% cell-survival rates. By means of statistical regression, 2250 genes were identified that showed statistically significant changes in expression after treatment with *N*-hydroxy-AABP; they included induced

stress-response genes such as members of the class of heat-shock proteins [Hsp40 homologue (DNAJ), Hsp70, Hsp105, and Hsp125], and metal regulatory transcription factor 1 (MTF1). Another gene whose expression was upregulated was the *XPA* gene, which encodes a DNA-damage recognition protein involved in excision repair. Several of the genes were also induced in TK6 cells treated with benzo[*a*]pyrene diol epoxide (Luo *et al.*, 2005; Ricicki *et al.*, 2006). The subsets of commonly regulated genes were indicative of a general cellular response to toxicity to these two classes of carcinogenic agents.

DNA adducts were formed in transitional cell carcinoma (TCC) cell lines from the huan urinary bladder expressing wild-type or mutant *TP53*, following exposure to *N*-hydroxy-ABP, *N*-hydroxy-AABP or *N*-acetoxy-4-acetylaminobiphenyl (*N*-OAc-AABP) (Swaminathan *et al.*, 2002). The major adduct in the cell lines was identified as dG-C8–4-ABP with all three chemicals. The number of adducts ranged from 0.1 to 20 per 10^6 nucleotides, with *N*-OAc-AABP yielding the highest adduct levels after 2 hours of treatment. Only *N*-OAc-AABP (5 μM) induced an apoptotic response, which was independent of the *TP53* status. However, the *TP53* status did affect repair rates, and the level of the dG-C8–4-ABP adduct was approximately 2-fold higher in TCC cells with mutant *TP53* than with wild-type *TP53*, at 24 hours post-treatment. The authors concluded that *TP53* could be modulating the repair of 4-ABP-DNA adducts in the human uroepithelial cells, and that unrepaired DNA damage that accumulates in TP53-deficient cells could cause an accumulation of mutation, increase genomic instability, and accelerate neoplastic progression.

4.3 Mechanistic considerations

Since biomonitoring was introduced to assess exposure to aromatic amines, 4-aminobiphenyl (4-ABP) was found in human samples. Originally the analysis of haemoglobin adducts pointed to cigarette smoke as a source. Recently, 4-ABP as well as *ortho*-toluidine and 2-naphthylamine were detected in the urine of smokers and non-smokers. The concentrations were significantly higher in smokers than in nonsmokers in all three cases, but it was concluded that 4-ABP is a general environmental contaminant (Riedel *et al.*, 2006). Looking for the sources, 4-nitrobiphenyl must be considered to contribute to the exposure. This applies not only to the assessment of haemoglobin adducts, but also to guanine-C8-ABP-adducts in DNA. The biomarkers correlate with three risk factors for bladder cancer: cigarette smoking, tobacco brand and slow-acetylator phenotype. DNA adducts have not only been demonstrated in human bladder tumours (Zayas *et al.*, 2007), bladder epithelium (Skipper & Tannenbaum, 1994) and in exfoliated urothelial cells (Talaska *et al.*, 1993), but also in mammary (Faraglia *et al.*, 2003) and other tissues (Cohen *et al.*, 2006; Saletta *et al.*, 2007).

The common activation product of aminobiphenyls is the *N*-hydroxy derivative. Studies in dogs indicate that *N*-oxidation to *N*-hydroxyaminobiphenyl takes place in the liver, from where the metabolite is transferred to the bladder. The hydroxylamine may be

further activated by conjugation to form sulfate or acetate. The conjugates are unstable and give rise to the nitrenium ion that adds to DNA and gives promutagenic DNA adducts. Modifying factors of DNA-adduct formation in the bladder are the pH of the urine and the frequency of urination (Kadlubar *et al.*, 1991). Although CYP1A2 is considered to be the enzyme primarily responsible for the oxidation of arylamines in the liver, experiments with *CYP1A2*-knockout mice show that this is not the case with 4-ABP (Kimura *et al.*, 1999; Shertzer *et al.*, 2002). The lack of CYP1A2 did not influence the decrease of the thiol SH-pool in the liver, did not interfere with the consumption of glutathione in red cells, and increased the formation of methaemoglobin (Shertzer *et al.*, 2002). This bears on the interpretation of individual susceptibility of workers exposed to 4-ABP. The activity of CYP1A2 varies by a factor of 60 in humans, and workers with high levels of active enzyme have been assumed to be more susceptible to developing bladder tumours than those with low levels of this enzyme. Another possible route of activation was suggested by the results of studies in *S. typhimurium* strain TA102. In this case 4-ABP, activated by liver S9, induced mutations through generation of reactive oxygen species (ROS) (Makena & Chung, 2007).

The formation of haemoglobin adducts and DNA adducts in tissues other than the bladder supports the view that the activated metabolite is not only available in the target tissue for tumour formation but throughout the organism, and factors other than bladder-specific activation must be responsible for the development of the bladder tumours.

In addition to the dG-C8-ABP adduct, the dG-*N*2-ABP adduct and the dA-C8-ABP adduct were observed (Beland & Kadlubar, 1985; Swaminathan & Hatcher, 2002). Although 4-ABP like 2-aminofluorene (2-AF) produces the typical guanine-C8-adduct, the mutation pattern of the two chemicals is different. dG-C8-AF produces both frameshift and base-substitution (G to T) mutations, and dG-C8-ABP only base-substitution (G to A) mutations, 2-AF being more mutagenic than ABP. These differences are considered to arise from the different conformational changes induced in DNA by aromatic amines, including conversion from the *anti*- to the *syn*-conformation (Beland *et al*., 1983; Beland & Kadlubar, 1985). So far a key to explain tissue specificity and individual susceptibility has not been found.

Gene-expression profiles were measured in the TK6 lymphoblastoid cell line after exposure to *N*-hydroxy-4-aminobiphenyl. The amount of the major dG-C8-ABP adduct was correlated with cell toxicity, mutation at the *TK* (thymidine kinase) and *HPRT* loci and changes in gene expression. The expression of 2250 genes was significantly altered. Five genes related to functions of cell survival and cell growth were downregulated; all the others were upregulated (Ricicki *et al.*, 2006). The results indicate a possible approach to relate gene-expression patterns with phenotypic markers, but also show the complexity of the cellular response to a chemical insult.

More recent results emphasize that the induction of mutations in the human genome may not be the only relevant genotoxic effect. Saletta *et al.* (2007) propose that gross chromosomal alterations rather than point mutations are responsible for the formation of bladder tumours. Chromosome aberrations and chromosomal instability are held to play a

central role. The induction of chromosomal instability can be carcinogen-specific. 4-ABP induces chromosomal instability (CIN) in genetically stable RT112 bladder cells, but no microsatellite instability (MIN). Conversely, *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG) produces MIN but not CIN in these cells. CIN is generally defined as the ability to gain and lose chromosomes, a property frequently observed in tumours, which makes them resistant to the toxic effects of carcinogens and gives them a selective advantage. However, the actual role of chromosomal instability in ABP-induced bladder cancer remains to be established (Saletta *et al.*, 2007).

The expanding knowledge about metabolic activation of 4-ABP and the causal relationship between DNA-adduct formation and mutagenesis stimulated the expectation that differences in tissues and species, as well as individual susceptibility can be explained on the basis of the pharmacokinetic behaviour. However, Beland and Kadlubar and others stated as early as 1985: "It is clear that adduct formation *per se* is not sufficient for tumour initiation and that other processes, such as error-prone repair and stimulation of cell replication, play an essential role." Even today, the tissue-specific effects of 4-ABP can neither be answered by specific mutations or mutation rate nor by selective advantage. After so many years of research about one of the first classified human carcinogens it still seems unlikely that a critical pathway can be found that leads from exposure to cell transformation and tumour development. However, the data collected thus far support a common mode of action for many aromatic amines, in which genotoxic effects and toxicity both have a role. This mode of action is defined as interaction with cellular regulation pathways. In their review, Cohen *et al.* (2006) call it "synergy of DNA reactivity and cell proliferation." The emphasis so far has been on DNA-reactivity. The role of toxicity and the underlying biochemistry and molecular biology has been neglected and is essentially unknown. Epidemiology and animal experiments support the classification of 4-ABP as carcinogenic to humans. The toxicological profile shows many properties typical for the group of carcinogenic aromatic amines and their common mode of action. Since 4-ABP and 4-nitrobiphenyl are present in the general environment, any further assessment requires more knowledge about the background exposure and the incremental contribution of specific sources.

5. Summary of Data Reported

5.1 Exposure data

4-Aminobiphenyl was formerly used as a rubber oxidant, as a dye intermediate and for the detection of sulfates. Currently it is only used for research purposes. Its production has been prohibited in the European Union since 1998 and ceased in the USA in the 1950s. 4-Aminobiphenyl is still produced in some countries and supplied to countries where it is no longer produced. 4-Aminobiphenyl may occur as a contaminant in 2-aminodiphenyl, in some cosmetic colour additives, in hair dyes and in the fungicide

diphenylamine. Occupational exposure may occur during production and use of the compound. The main sources of exposure for the general population are tobacco smoking and environmental tobacco smoke, although the use of hair dye contaminated with 4-aminobiphenyl is also a potential source of exposure.

5.2 Human carcinogenicity data

Excesses of bladder-cancer risk have been found in a case series and a cohort study in workers producing and using many chemicals including 4-aminobiphenyl. The most marked excess of bladder cancer occurred in workers exposed to 4-aminobiphenyl. Chance, bias and confounding from other chemical exposures or smoking offer no credible explanation for these increased risks.

5.3 Animal carcinogenicity data

4-Aminobiphenyl was tested for carcinogenicity by oral administration in mice, rabbits and dogs, and by subcutaneous administration in rats. Following its oral administration, it induced bladder papillomas and carcinomas in rabbits and dogs, and neoplasms at various sites in mice, including dose-related increases in the incidences of angiosarcomas, hepatocellular tumours and bladder carcinomas. When administered subcutaneously to rats, it induced tumours of the intestine. When administered by intraperitoneal injection to newborn mice or *CYP1A2-null* mice, it caused an increase in the incidence of liver tumours.

5.4 Other relevant data

The bioactivation of 4-aminobiphenyl (4-ABP) *in vitro*, in experimental animals and in humans is well documented. The genotoxic metabolite that binds to DNA is *N*-hydroxy-4-ABP; its oxidised derivative 4-nitrosobiphenyl binds to haemoglobin. Human CYP1A2 displays the highest catalytic activity of the P450 enzymes for *N*-oxidation of 4-ABP; however, extrahepatic CYPs, peroxidases and prostaglandin H synthase (PHS) can catalyse bioactivation within target sites. *N*-acetylation is an important detoxification pathway of 4-ABP. Some epidemiological investigations on aromatic amine exposure have reported that individuals who are slow NAT2 *N*-acetylators are at elevated risk for bladder cancer, and 4-ABP has been implicated as one of the causal agents. More recent studies have established that NAT2 is the enzyme responsible for the observed differences. Elevated levels of 4-ABP haemoglobin- or DNA-adducts have been detected in these populations and support a role for 4-ABP in urinary bladder cancer and possibly other cancers.

6. Evaluation

6.1 Cancer in humans

There is *sufficient evidence* in humans for the carcinogenicity of 4-aminobiphenyl. 4-Aminobiphenyl causes bladder cancer in humans.

6.2 Cancer in experimental animals

There is *sufficient evidence* in experimental animals for the carcinogenicity of 4-aminobiphenyl.

6.3 Overall evaluation

4-Aminobiphenyl is *carcinogenic to humans (Group 1)*.

7. References

ACGIH (2001) Documentation of the Threshold Limit Values and Biological Exposure Indices, 7th Ed., American Conference of Governmental Industrial Hygienists. Cincinnati, OH.

Adris P, Chung KT (2006). Metabolic activation of bladder procarcinogens, 2-aminofluorene, 4-aminobiphenyl, and benzidine by Pseudomonas aeruginosa and other human endogenous bacteria. *Toxicol In Vitro*, 20:367–374. doi:10.1016/j.tiv.2005.08.017 PMID:16203120

Akyüz M (2007). Simultaneous determination of aliphatic and aromatic amines in indoor and outdoor air samples by gas chromatography-mass spectrometry. *Talanta*, 71:486–492. doi:10.1016/j.talanta.2006.10.028 PMID:19071331

Akyüz M, Ata S (2008). Determination of aromatic amines in hair dye and henna samples by ion-pair extraction and gas chromatography-mass spectrometry. *J Pharm Biomed Anal*, 47:68–80. doi:10.1016/j.jpba.2007.12.011 PMID:18280687

Ambrosone CB, Abrams SM, Gorlewska-Roberts K, Kadlubar FF (2007). Hair dye use, meat intake, and tobacco exposure and presence of carcinogen-DNA adducts in exfoliated breast ductal epithelial cells. *Arch Biochem Biophys*, 464:169–175. doi:10.1016/j.abb.2007.05.018 PMID:17601487

Anderson KE, Hammons GJ, Kadlubar FF *et al.* (1997). Metabolic activation of aromatic amines by human pancreas. *Carcinogenesis*, 18:1085–1092. doi:10.1093/carcin/18.5.1085 PMID:9163700

Bafana A, Devi SS, Krishnamurthi K, Chakrabarti T (2007). Kinetics of decolourisation and biotransformation of direct black 38 by C. hominis and P. stutzeri. *Appl Microbiol Biotechnol*, 74:1145–1152. doi:10.1007/s00253-006-0751-5 PMID:17318544

Beland FA, Beranek DT, Dooley KL *et al.* (1983). Arylamine-DNA adducts in vitro and in vivo: their role in bacterial mutagenesis and urinary bladder carcinogenesis. *Environ Health Perspect*, 49:125–134. doi:10.2307/3429589 PMID:6339219

Beland FA, Kadlubar FF (1985). Formation and persistence of arylamine DNA adducts in vivo. *Environ Health Perspect*, 62:19–30. doi:10.2307/3430089 PMID:4085422

Besaratinia A, Bates SE, Pfeifer GP (2002). Mutational signature of the proximate bladder carcinogen N-hydroxy-4-acetylaminobiphenyl: inconsistency with the p53 mutational spectrum in bladder cancer. *Cancer Res*, 62:4331–4338. PMID:12154037

Besaratinia A, Besaratinia A, Van Straaten HW *et al.* (2000). Immunoperoxidase detection of 4-aminobiphenyl- and polycyclic aromatic hydrocarbons-DNA adducts in induced sputum of smokers and non-smokers. *Mutat Res*, 468:125–135. PMID:10882891

Beyerbach A, Rothman N, Bhatnagar VK *et al.* (2006). Hemoglobin adducts in workers exposed to benzidine and azo dyes. *Carcinogenesis*, 27:1600–1606. doi:10.1093/carcin/bgi362 PMID:16497705

Block NL, Sigel MM, Lynne CM *et al.* (1978). The initiation, progress, and diagnosis of dog bladder cancer induced by 4-aminobiphenyl. *Invest Urol*, 16:50–54. PMID:689839

Bonser GM (1962) Precancerous changes in the urinary bladder.1962. Perugia: 435–439.

Bonser GM, Bradshaw L, Clayson DB, Jull JW (1956). A further study of the carcinogenic properties of ortho hydroxy-amines and related compounds by bladder implantation in the mouse. *Br J Cancer*, 10:539–546. PMID:13396105

Bookland EA, Reznikoff CA, Lindstrom M, Swaminathan S (1992b). Induction of thioguanine-resistant mutations in human uroepithelial cells by 4-aminobiphenyl and its N-hydroxy derivatives. *Cancer Res*, 52:1615–1621. PMID:1311636

Bookland EA, Swaminathan S, Oyasu R *et al.* (1992a). Tumorigenic transformation and neoplastic progression of human uroepithelial cells after exposure in vitro to 4-aminobiphenyl or its metabolites. *Cancer Res*, 52:1606–1614. PMID:1311635

Bryant MS, Vineis P, Skipper PL, Tannenbaum SR (1988). Hemoglobin adducts of aromatic amines: associations with smoking status and type of tobacco. *Proc Natl Acad Sci USA*, 85:9788–9791. doi:10.1073/pnas.85.24.9788 PMID:3200858

Butler MA, Iwasaki M, Guengerich FP, Kadlubar FF (1989). Human cytochrome P-450PA (P-450IA2), the phenacetin O-deethylase, is primarily responsible for the hepatic 3-demethylation of caffeine and N-oxidation of carcinogenic arylamines. *Proc Natl Acad Sci USA*, 86:7696–7700. doi:10.1073/pnas.86.20.7696 PMID:2813353

Butler MA, Lang NP, Young JF *et al.* (1992). Determination of CYP1A2 and NAT2 phenotypes in human populations by analysis of caffeine urinary metabolites. *Pharmacogenetics*, 2:116–127. doi:10.1097/00008571-199206000-00003 PMID:1306111

Chemical Sources International (2008) ChemSources database. [http://db2.chemsources.com]

Chen SC, Kao CM, Huang MH *et al.* (2003). Assessment of genotoxicity of benzidine and its structural analogues to human lymphocytes using comet assay. *Toxicol Sci*, 72:283–288. doi:10.1093/toxsci/kfg026 PMID:12660362

Chen T, Mittelstaedt RA, Beland FA *et al.* (2005). 4-Aminobiphenyl induces liver DNA adducts in both neonatal and adult mice but induces liver mutations only in neonatal mice. *Int J Cancer*, 117:182–187. doi:10.1002/ijc.21173 PMID:15880493

Chiang TA, Pei-Fen W, Ying LS *et al.* (1999). Mutagenicity and aromatic amine content of fumes from heated cooking oils produced in Taiwan. *Food Chem Toxicol*, 37:125–134. doi:10.1016/S0278-6915(98)00081-7 PMID:10227736

Chou HC, Lang NP, Kadlubar FF (1995). Metabolic activation of N-hydroxy arylamines and N-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res*, 55:525–529. PMID:7834621

Chung KT, Chen SC, Wong TY *et al.* (2000). Mutagenicity studies of benzidine and its analogs: structure-activity relationships. *Toxicol Sci*, 56:351–356. doi:10.1093/toxsci/56.2.351 PMID:10910993

Clayson DB, Lawson TA, Pringle JA (1967). The carcinogenic action of 2-aminodiphenylene oxide and 4-aminodiphenyl on the bladder and liver of the C57 X IF mouse. *Br J Cancer*, 21:755–762. PMID:6074696

Clayson DB, Lawson TA, Santana S, Bonser GM (1965). Correlation between the chemical induction of hyperplasia and of malignancy in the bladder epithelium. *Br J Cancer*, 19:297–310. PMID:14316204

Cohen SM, Boobis AR, Meek ME *et al.* (2006). 4-Aminobiphenyl and DNA reactivity: case study within the context of the 2006 IPCS Human Relevance Framework for Analysis of a cancer mode of action for humans. *Crit Rev Toxicol*, 36:803–819. doi:10.1080/10408440600977651 PMID:17118730

Collins JJ, Strauss ME, Riordan SG (1999). Mortalities of workers at the Nitro plant with exposure to 2-mercaptobenzothialzole. *Occup Environ Med*, 56:667–671. doi:10.1136/oem.56.10.667 PMID:10658544

Culp SJ, Roberts DW, Talaska G *et al.* (1997). Immunochemical, 32P-postlabeling, and GC/MS detection of 4-aminobiphenyl-DNA adducts in human peripheral lung in relation to metabolic activation pathways involving pulmonary N-oxidation, conjugation, and peroxidation. *Mutat Res*, 378:97–112. PMID:9288889

Dang LN, McQueen CA (1999). Mutagenicity of 4-aminobiphenyl and 4-acetylaminobiphenyl in Salmonella typhimurium strains expressing different levels of N-acetyltransferase. *Toxicol Appl Pharmacol*, 159:77–82. doi:10.1006/taap.1999.8727 PMID:10495770

Deichmann WB, MacDonald WE (1968). The non-carcinogenicity of a single dose of 4-aminobiphenyl in the dog. *Food Cosmet Toxicol*, 6:143–146. doi:10.1016/0015-6264(68)90195-8 PMID:5671147

Deichmann WB, Radomski JL, Anderson WA *et al.* (1958). The carcinogenic action of p-aminobiphenyl in the dog; final report. *Ind Med Surg*, 27:25–26. PMID:13491120

Deichmann WB, Radomski JL, Glass E *et al.* (1965). Synergism among oral carcinogens. 3. simultaneous feeding of four bladder carcinogens to dogs. *Ind Med Surg*, 34:640–649. PMID:14334179

Doerge DR, Churchwell MI, Marques MM, Beland FA (1999). Quantitative analysis of 4-aminobiphenyl-C8-deoxyguanosyl DNA adducts produced in vitro and in vivo using HPLC-ES-MS. *Carcinogenesis*, 20:1055–1061. doi:10.1093/carcin/20.6.1055 PMID:10357788

Doherty L (2005). [GC/MS analysis of forbidden amine carcinogens in 20 textiles and consumption good, and amine carcinogens appeared in environmental monitoring of textile treatment wastewater.] *Huanjing Huaxue*, 24:622–625.

Dooley KL, Von Tungeln LS, Bucci T *et al.* (1992). Comparative carcinogenicity of 4-aminobiphenyl and the food pyrolysates, Glu-P-1, IQ, PhIP, and MeIQx in the neonatal B6C3F1 male mouse. *Cancer Lett*, 62:205–209. doi:10.1016/0304-3835(92)90097-F PMID:1596864

European Commission (1998) Council Directive 98/24/EC of 7 April 1998 on the protection of the health and safety of workers from the risks related to chemical agents at work (fourteenth individual Directive within the meaning of Article 16(1) of Directive 89/391/EEC)

European Commission (2002) Directive 2002/61/EC of the European Parliament and of the Council of 19 July 2002 amending for the nineteenth time Council Directive 76/769/EEC relating to restrictions on the marketing and use of certain dangerous substances and preparations (azocolourants)

European Commission (2004a) Directive 2004/37/EC of the European Parliament and of the council of 29 April 2004 on the protection of workers from the risks related to exposure to carcinogens or mutagens at work (Sixth individual Directive within the meaning of Article 16(1) of Council Directive 89/391/EEC) (codified version)

European Commission (2004b) Commission Directive 2004/93/EC of 21 September 2004 amending Council Directive 76/768/EEC for the purpose of adapting its Annexes II and III to technical progress.

European Economic Community (1989) Council Directive 89/677/EEC of 21 December 1989 amending for the eighth time Directive 76/769/EEC on the approximation of the laws, regulations and administrative provisions of the member states relating to restrictions on the marketing and use of certain dangerous substances and preparations.

Faraglia B, Chen SY, Gammon MD *et al.* (2003). Evaluation of 4-aminobiphenyl-DNA adducts in human breast cancer: the influence of tobacco smoke. *Carcinogenesis*, 24:719–725. doi:10.1093/carcin/bgg013 PMID:12727801

FDA (Food and Drug Administration) (1985). US *Fed Regist*, 50:35774–35790.

FDA (Food and Drug Administration) (1986). US *Fed Regist*, 51:41765–41783.

FDA (Food and Drug Administration) (1988). US *Fed Regist*, 53:33110–33121.

FDA (Food and Drug Administration) (2009) US Code of Federal Regulations, Title 21, Volume 1, Chapter 1, Subchapter A, Part 74 : Listing of Color Additives Subject to Certification [online version]

Feng Z, Hu W, Rom WN *et al.* (2002a). 4-aminobiphenyl is a major etiological agent of human bladder cancer: evidence from its DNA binding spectrum in human p53 gene. *Carcinogenesis*, 23:1721–1727. doi:10.1093/carcin/23.10.1721 PMID:12376482

Feng Z, Hu W, Rom WN *et al.* (2002b). N-hydroxy-4-aminobiphenyl-DNA binding in human p53 gene: sequence preference and the effect of C5 cytosine methylation. *Biochemistry*, 41:6414–6421. doi:10.1021/bi020093s PMID:12009904

Flammang TJ, Yamazoe Y, Benson RW *et al.* (1989). Arachidonic acid-dependent peroxidative activation of carcinogenic arylamines by extrahepatic human tissue microsomes. *Cancer Res*, 49:1977–1982. PMID:2495173

Fletcher K, Tinwell H, Ashby J (1998). Mutagenicity of the human bladder carcinogen 4-aminobiphenyl to the bladder of MutaMouse transgenic mice. *Mutat Res*, 400:245–250. PMID:9685665

Gan J, Skipper PL, Gago-Dominguez M *et al.* (2004). Alkylaniline-hemoglobin adducts and risk of non-smoking-related bladder cancer. *J Natl Cancer Inst*, 96:1425–1431. doi:10.1093/jnci/djh274 PMID:15467031

Garrigós MC, Reche F, Marín ML, Jiménez A (2002). Determination of aromatic amines formed from azo colorants in toy products. *J Chromatogr A*, 976:309–317. doi:10.1016/S0021-9673(02)01162-7 PMID:12462623

Garrigós MC, Reche F, Pernías K *et al.* (1998). Determination of some aromatic amines in finger-paints for children's use by supercritical fluid extraction combined with gas chromatography. *J Chromatogr A*, 819:259–266. doi:10.1016/S0021-9673(98)00432-4 PMID:9781420

Garrigós MC, Reche F, Pernías K, Jiménez A (2000). Optimization of parameters for the analysis of aromatic amines in finger-paints. *J Chromatogr A*, 896:291–298. doi:10.1016/S0021-9673(00)00742-1 PMID:11093664

GESTIS (2007) International limit values for chemical agents, BG Institute for Occupational Safety and Health, Sankt Augustin. Available at: http://www.dguv.de/fa/en/gestis/limit_values/index.jsp (accessed 20/04/10)

Gorlewska-Roberts KM, Teitel CH, Lay JO Jr *et al.* (2004). Lactoperoxidase-catalyzed activation of carcinogenic aromatic and heterocyclic amines. *Chem Res Toxicol*, 17:1659–1666. doi:10.1021/tx049787n PMID:15606142

Gorrod JW, Carter RL, Roe FJ (1968). Induction of hepatomas by 4-aminobiphenyl and three of its hydroxylated derivatives administered to newborn mice. *J Natl Cancer Inst*, 41:403–410. PMID:4299538

Green LC, Skipper PL, Turesky RJ *et al.* (1984). In vivo dosimetry of 4-aminobiphenyl in rats via a cysteine adduct in hemoglobin. *Cancer Res*, 44:4254–4259. PMID:6467185

Grimmer G, Dettbarn G, Seidel A, Jacob J (2000). Detection of carcinogenic aromatic amines in the urine of non-smokers. *Sci Total Environ*, 247:81–90. doi:10.1016/S0048-9697(99)00471-4 PMID:10721145

Hammond SK, Sorensen G, Youngstrom R, Ockene JK (1995). Occupational exposure to environmental tobacco smoke. *JAMA*, 274:956–960. doi:10.1001/jama.274.12.956 PMID:7674526

Hatcher JF, Swaminathan S (2002). Identification of N-(deoxyguanosin-8-yl)-4-azobiphenyl by (32)P-postlabeling analyses of DNA in human uroepithelial cells exposed to proximate metabolites of the environmental carcinogen 4-aminobiphenyl. *Environ Mol Mutagen*, 39:314–322. doi:10.1002/em.10079 PMID:12112383

Hauri U, Lutolf B, Schlegel U *et al.* (2005). Determination of carcinogenic aromatic amines in dyes, cosmetics, finger paints and inks for pens and tattoos with LC/MS. *Mitt Lebensm Hyg*, 96:321–335.

Hix C, Oglesby L, MacNair P *et al.* (1983). Bovine bladder and liver cell and homogenate-mediated mutagenesis of Salmonella typhimurium with aromatic amines. *Carcinogenesis*, 4:1401–1407. doi:10.1093/carcin/4.11.1401 PMID:6357520

Hoffmann D, Djordjevic MV, Hoffmann I (1997). The changing cigarette. *Prev Med*, 26:427–434. doi:10.1006/pmed.1997.0183 PMID:9245661

HSDB (2009) Hazardous Substances Data Bank. 4-Aminobiphenyl. National Library of Medicine. Last updated: 12/08/2009. HSDB and search CAS number. Accessed: 08/03/10. [http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB]

IARC (1987). Overall evaluations of carcinogenicity: an updating of IARC Monographs volumes 1 to 42. *IARC Monogr Eval Carcinog Risks Hum Suppl*, 7:1–440. PMID:3482203

IARC (2004). Tobacco smoke and involuntary smoking. *IARC Monogr Eval Carcinog Risks Hum*, 83:1–1438. PMID:15285078

Japan Society for Occupational Health (2007). Recommendation of occupational exposure limits (2007–2008). *J Occup Health*, 49:328–344 http://joh.med.uoeh-u.ac.jp/oel/index.html. PMID:17708058

Jiang X, Yuan JM, Skipper PL *et al.* (2007). Environmental tobacco smoke and bladder cancer risk in never smokers of Los Angeles County. *Cancer Res*, 67:7540–7545. doi:10.1158/0008-5472.CAN-07-0048 PMID:17671226

Josephy PD (1996). The role of peroxidase-catalyzed activation of aromatic amines in breast cancer. *Mutagenesis*, 11:3–7. doi:10.1093/mutage/11.1.3 PMID:8671708

Kadlubar FF, Dooley KL, Teitel CH *et al.* (1991). Frequency of urination and its effects on metabolism, pharmacokinetics, blood hemoglobin adduct formation, and liver and urinary bladder DNA adduct levels in beagle dogs given the carcinogen 4-aminobiphenyl. *Cancer Res*, 51:4371–4377. PMID:1868460

Kiese M (1966). The biochemical production of ferrihemoglobin-forming derivatives from aromatic amines, and mechanisms of ferrihemoglobin formation. *Pharmacol Rev*, 18:1091–1161. PMID:5343079

Kim D, Kadlubar FF, Teitel CH, Guengerich FP (2004). Formation and reduction of aryl and heterocyclic nitroso compounds and significance in the flux of hydroxylamines. *Chem Res Toxicol*, 17:529–536. doi:10.1021/tx034267y PMID:15089095

Kimura S, Kawabe M, Ward JM *et al.* (1999). CYP1A2 is not the primary enzyme responsible for 4-aminobiphenyl-induced hepatocarcinogenesis in mice. *Carcinogenesis*, 20:1825–1830. doi:10.1093/carcin/20.9.1825 PMID:10469630

Koss LG, Melamed MR, Kelly E (1969). Further cytologic and histologic studies of bladder lesions in workers exposed to para-aminodiphenyl: progress report. *J Natl Cancer Inst*, 43:233–243. PMID:5796385

Koss LG, Melamed MR, Ricci A *et al.* (1965). Carcinogenesis in the human urinary bladder: observations after exposure to para-aminodiphenyl. *N Engl J Med*, 272:767–770. PMID:14263636

Lakshmi VM, Hsu FF, Zenser TV (2003). Transformation and activation of benzidine by oxidants of the inflammatory response. *Chem Res Toxicol*, 16:367–374. doi:10.1021/tx0200966 PMID:12641437

Lide DR, editor (2008) *CRC Handbook of Chemistry and Physics*, 98th Ed., Boca Raton, FL, CRC Press, p. 3–16.

Lin D, Lay JO Jr, Bryant MS *et al.* (1994). Analysis of 4-aminobiphenyl-DNA adducts in human urinary bladder and lung by alkaline hydrolysis and negative ion gas chromatography-mass spectrometry. *Environ Health Perspect*, 102 Suppl 6;11–16. doi:10.2307/3432144 PMID:7889831

Lord PG, Hardaker KJ, Loughlin JM *et al.* (1992). Point mutation analysis of ras genes in spontaneous and chemically induced C57Bl/10J mouse liver tumours. *Carcinogenesis*, 13:1383–1387. doi:10.1093/carcin/13.8.1383 PMID:1499088

Luo W, Fan W, Xie H *et al.* (2005). Phenotypic anchoring of global gene expression profiles induced by N-hydroxy-4-acetylaminobiphenyl and benzo[a]pyrene diol epoxide reveals correlations between expression profiles and mechanism of toxicity. *Chem Res Toxicol*, 18:619–629. doi:10.1021/tx049828f PMID:15833022

MAK (2007) *List of MAK and BAT Values 2007*. Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area, Report No. 43, Deutsche Forschungsgemeinschaft, Weinheim, Wiley-VCH Verlag GmbH & Co. KGaA.

Makena PS, Chung KT (2007). Evidence that 4-aminobiphenyl, benzidine, and benzidine congeners produce genotoxicity through reactive oxygen species. *Environ Mol Mutagen*, 48:404–413. doi:10.1002/em.20288 PMID:17370336

Manjanatha MG, Li EE, Fu PP, Heflich RH (1996). H- and K-ras mutational profiles in chemically induced liver tumors from B6C3F1 and CD-1 mice. *J Toxicol Environ Health*, 47:195–208. doi:10.1080/009841096161898 PMID:8598575

Melamed MR (1972). Diagnostic cytology of urinary tract carcinoma. A review of experience with spontaneous and carcinogen induced tumors in man. *Eur J Cancer*, 8:287–292. PMID:5074773

Melamed MR, Koss LG, Ricci A, Whitmore WF (1960). Cytohistological observations on developing carcinoma of the urinary bladder in man. *Cancer*, 13:67–74. doi:10.1002/1097-0142(196001/02)13:1<67::AID-CNCR2820130112>3.0.CO;2-K.

Melchior WB Jr, Marques MM, Beland FA (1994). Mutations induced by aromatic amine DNA adducts in pBR322. *Carcinogenesis*, 15:889–899. doi:10.1093/carcin/15.5.889 PMID:8200092

Melick WF, Escue HM, Naryka JJ *et al.* (1955). The first reported cases of human bladder tumors due to a new carcinogen-xenylamine. *J Urol*, 74:760–766. PMID:13278983

Melick WF, Naryka JJ, Kelly RE (1971). Bladder cancer due to exposure to para-aminobiphenyl: a 17-year followup. *J Urol*, 106:220–226. PMID:5099312

Minchin RF, Reeves PT, Teitel CH *et al.* (1992). N-and O-acetylation of aromatic and heterocyclic amine carcinogens by human monomorphic and polymorphic acetyltransferases expressed in COS-1 cells. *Biochem Biophys Res Commun*, 185:839–844. doi:10.1016/0006-291X(92)91703-S PMID:1627140

Nakajima M, Itoh M, Sakai H *et al.* (2006). CYP2A13 expressed in human bladder metabolically activates 4-aminobiphenyl. *Int J Cancer*, 119:2520–2526. doi:10.1002/ijc.22136 PMID:16988941

NIOSH (2005) NIOSH Pocket Guide to Chemical Hazards, NIOSH Publication No. 2005–149 http://www.cdc.gov/niosh/npg/nengapdx.html#a

NTP (2005) 4-Aminobiphenyl CAS No. 92–67–1, *Report on Carcinogens*, Eleventh Edition; U.S. Department of Health and Human Services, Public Health Service, National Toxicology Program http://ntp.niehs.nih.gov/ntp/roc/eleventh/profiles/s010amin.pdf

O'Neil MJ, editor (2006) *The Merck Index*, 14th Ed., Whitehouse Station, NJ, Merck & Co., Inc., p. 204.

Oda Y (2004). Analysis of the involvement of human N-acetyltransferase 1 in the genotoxic activation of bladder carcinogenic arylamines using a SOS/umu assay system. *Mutat Res*, 554:399–406. PMID:15450435

Oglesby LA, Hix C, Snow L *et al.* (1983). Bovine bladder urothelial cell activation of carcinogens to metabolites mutagenic to Chinese hamster V79 cells and Salmonella typhimurium. *Cancer Res*, 43:5194–5199. PMID:6352016

Olek M (1988). Determination of diphenylamine residues in apples, and 4-aminobiphenyl residues in diphenylamine, by high-performance liquid chromatography and electrochemical detection. *J Chromatogr A*, 447:421–425. doi:10.1016/S0021-9673(01)91505-5.

Olivier M, Eeles R, Hollstein M *et al.* (2002). The IARC TP53 database: new online mutation analysis and recommendations to users. *Hum Mutat*, 19:607–614. doi:10.1002/humu.10081 PMID:12007217

Parsons BL, Beland FA, Von Tungeln LS *et al.* (2005). Levels of 4-aminobiphenyl-induced somatic H-ras mutation in mouse liver DNA correlate with potential for liver tumor development. *Mol Carcinog*, 42:193–201. doi:10.1002/mc.20083 PMID:15761837

Patrianakos C, Hoffmann D (1979). Chemical studies on tobacco smoke. LXIV. On the analysis of aromatic amines in cigarette smoke. *J Anal Toxicol*, 3:150–154.

Pohanish RP (2008) *Sittig's Handbook of Toxic and Hazardous Chemicals and Carcinogens*, 5th Ed., Vol. 1:A-H, Norwich, NY, William Andrew Inc., pp. 125–127.

Poirier MC, Fullerton NF, Smith BA, Beland FA (1995). DNA adduct formation and tumorigenesis in mice during the chronic administration of 4-aminobiphenyl at multiple dose levels. *Carcinogenesis*, 16:2917–2921. doi:10.1093/carcin/16.12.2917 PMID:8603464

Probst-Hensch NM, Bell DA, Watson MA *et al.* (2000). N-acetyltransferase 2 phenotype but not NAT1*10 genotype affects aminobiphenyl-hemoglobin adduct levels. *Cancer Epidemiol Biomarkers Prev*, 9:619–623. PMID:10868698

Radomski JL, Glass EM, Deichmann WB (1971). Transitional cell hyperplasia in the bladders of dogs fed DL-tryptophan. *Cancer Res*, 31:1690–1694. PMID:5121671

Radomski JL, Radomski T, MacDonald WE (1977). Cocarcinogenic interaction between D,L-tryptophan and 4-aminobiphenyl or 2-naphthylamine in dogs. *J Natl Cancer Inst*, 58:1831–1834. PMID:864760

Reilly DA (1967). The determination of 4-aminobiphenyl in refined aniline. *Analyst (Lond)*, 92:642–644. doi:10.1039/an9679200642.

Richfield-Fratz N, Bailey JE Jr, Bailey CJ (1985). Determination of unsulfonated aromatic amines in FD&C Yellow No. 6 by the diazotization and coupling procedure followed by reversed-phase high-performance liquid chromatography. *J Chromatogr A*, 331:109–123. doi:10.1016/0021-9673(85)80012-1.

Ricicki EM, Luo W, Fan W *et al.* (2006). Quantification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl adducts in human lymphoblastoid TK6 cells dosed with N-hydroxy-4-acetylaminobiphenyl and their relationship to mutation, toxicity, and gene expression profiling. *Anal Chem*, 78:6422–6432. doi:10.1021/ac0607360 PMID:16970317

Ricicki EM, Soglia JR, Teitel C *et al.* (2005). Detection and quantification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl adducts in human pancreas tissue using capillary liquid chromatography-microelectrospray mass spectrometry. *Chem Res Toxicol*, 18:692–699. doi:10.1021/tx0496921 PMID:15833029

Riedel K, Scherer G, Engl J *et al.* (2006). Determination of three carcinogenic aromatic amines in urine of smokers and nonsmokers. *J Anal Toxicol*, 30:187–195. PMID:16803653

Ringe D, Turesky RJ, Skipper PL, Tannenbaum SR (1988). Structure of the single stable hemoglobin adduct formed by 4-aminobiphenyl in vivo. *Chem Res Toxicol*, 1:22–24. doi:10.1021/tx00001a003 PMID:2979706

Saletta F, Matullo G, Manuguerra M *et al.* (2007). Exposure to the tobacco smoke constituent 4-aminobiphenyl induces chromosomal instability in human cancer cells. *Cancer Res*, 67:7088–7094. doi:10.1158/0008-5472.CAN-06-4420 PMID:17671175

Sarkar M, Stabbert R, Kinser RD *et al.* (2006). CYP1A2 and NAT2 phenotyping and 3-aminobiphenyl and 4-aminobiphenyl hemoglobin adduct levels in smokers and non-smokers. *Toxicol Appl Pharmacol*, 213:198–206. doi:10.1016/j.taap.2005.11.003 PMID:16405939

Schieferstein GJ, Littlefield NA, Gaylor DW *et al.* (1985). Carcinogenesis of 4-aminobiphenyl in BALB/cStCrlfC3Hf/Nctr mice. *Eur J Cancer Clin Oncol*, 21:865–873. doi:10.1016/0277-5379(85)90227-5 PMID:2995043

Shertzer HG, Dalton TP, Talaska G, Nebert DW (2002). Decrease in 4-aminobiphenyl-induced methemoglobinemia in Cyp1a2(-/-) knockout mice. *Toxicol Appl Pharmacol*, 181:32–37. doi:10.1006/taap.2002.9398 PMID:12030840

Shimada T, Hayes CL, Yamazaki H *et al.* (1996). Activation of chemically diverse procarcinogens by human cytochrome P-450 1B1. *Cancer Res*, 56:2979–2984. PMID:8674051

Skipper PL, Tannenbaum SR (1994). Molecular dosimetry of aromatic amines in human populations. *Environ Health Perspect*, 102 Suppl 6;17–21. doi:10.2307/3432145 PMID:7889842

Swaminathan S, Hatcher JF (2002). Identification of new DNA adducts in human bladder epithelia exposed to the proximate metabolite of 4-aminobiphenyl using 32P-postlabeling method. *Chem Biol Interact*, 139:199–213. doi:10.1016/S0009-2797(01)00300-3 PMID:11823007

Swaminathan S, Torino JL, Burger MS (2002). Human urinary bladder epithelial cells lacking wild-type p53 function are deficient in the repair of 4-aminobiphenyl-DNA adducts in genomic DNA. *Mutat Res*, 499:103–117. PMID:11804609

Talaska G, al-Juburi AZ, Kadlubar FF (1991). Smoking related carcinogen-DNA adducts in biopsy samples of human urinary bladder: identification of N-(deoxyguanosin-8-yl)-4-aminobiphenyl as a major adduct. *Proc Natl Acad Sci USA*, 88:5350–5354. doi:10.1073/pnas.88.12.5350 PMID:2052611

Talaska G, Schamer M, Skipper P *et al.* (1993). Carcinogen-DNA adducts in exfoliated urothelial cells: techniques for noninvasive human monitoring. *Environ Health Perspect*, 99:289–291. doi:10.2307/3431503 PMID:8319646

Tsuneoka Y, Dalton TP, Miller ML *et al.* (2003). 4-aminobiphenyl-induced liver and urinary bladder DNA adduct formation in Cyp1a2(-/-) and Cyp1a2(+/+) mice. *J Natl Cancer Inst*, 95:1227–1237. doi:10.1093/jnci/djg025 PMID:12928348

Turesky RJ, Constable A, Richoz J *et al.* (1998). Activation of heterocyclic aromatic amines by rat and human liver microsomes and by purified rat and human cytochrome P450 1A2. *Chem Res Toxicol*, 11:925–936. doi:10.1021/tx980022n PMID:9705755

Turesky RJ, Freeman JP, Holland RD *et al.* (2003). Identification of aminobiphenyl derivatives in commercial hair dyes. *Chem Res Toxicol*, 16:1162–1173. doi:10.1021/tx030029r PMID:12971805

UN/UNEP/FAO (2007) Chemical Review Committee (CRC) 1. Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade. Notifications of Final Regulatory Actions by chemical name. [http://www.pic.int/home.php?type=b&id=7&sid=18&tid=42]

Verghis SB, Essigmann JM, Kadlubar FF *et al.* (1997). Specificity of mutagenesis by 4-aminobiphenyl: mutations at G residues in bacteriophage M13 DNA and G–>C transversions at a unique dG(8-ABP) lesion in single-stranded DNA. *Carcinogenesis*, 18:2403–2414. doi:10.1093/carcin/18.12.2403 PMID:9450488

Verschueren K (2001) *Handbook of Environmental Data on Organic Chemicals*, 4th Ed., New York, John Wiley & Sons, Inc., pp. 166–167.

Von Tungeln LS, Bucci TJ, Hart RW *et al.* (1996). Inhibitory effect of caloric restriction on tumorigenicity induced by 4-aminobiphenyl and 2-amino-1-methyl-6-phenylimidazo-[4,5-

b]pyridine (PhIP) in the CD1 newborn mouse bioassay. *Cancer Lett*, 104:133–136. doi:10.1016/0304-3835(96)04232-2 PMID:8665480

Walpole AL, Williams MH, Roberts DC (1952). The carcinogenic action of 4-aminodiphenyl and 3:2′-dimethyl-4-amino-diphenyl. *Br J Ind Med*, 9:255–263. PMID:12987579

Walpole AL, Williams MH, Roberts DC (1954). Tumours of the urinary bladder in dogs after ingestion of 4-aminodiphenyl. *Br J Ind Med*, 11:105–109. PMID:13149742

Wang LY, Chen CJ, Zhang YJ *et al.* (1998). 4-Aminobiphenyl DNA damage in liver tissue of hepatocellular carcinoma patients and controls. *Am J Epidemiol*, 147:315–323. PMID:9482507

Wise RW, Zenser TV, Kadlubar FF, Davis BB (1984). Metabolic activation of carcinogenic aromatic amines by dog bladder and kidney prostaglandin H synthase. *Cancer Res*, 44:1893–1897. PMID:6424934

Zack JA, Gaffey WR (1983). A mortality study of workers employed at the Monsanto Company plant in Nitro, West Virginia. *Environ Sci Res*, 26:575–591.

Zayas B, Stillwell SW, Wishnok JS *et al.* (2007). Detection and quantification of 4-ABP adducts in DNA from bladder cancer patients. *Carcinogenesis*, 28:342–349. doi:10.1093/carcin/bgl142 PMID:16926175